REMARKS

I. General Remarks

At the time of the Office Action, claims 1-5, 7 and 19-26 were pending and at issue. Claims 1 and 2 have been amended to insert a comma between hydrogen and halogen in the definition of R_4. Claim 3 has been amended as suggested by the Examiner to recite piperidinyl and homopiperidinyl instead of pieridino and homopiperidino. Claim 4 has been rewritten in independent form. Claims 19-21 and 25 have been amended to recite "an effective amount" instead of a VR1 antagonisticly effective amount. Claim 25 has also been amended to to recite "pain associated with a disease or disorder" as opposed to a disease or disorder associated with pain. Claim 26 has been amended consistent with claim 25 and to correct a clerical error No new matter has been added by this amendment.

Applicants thank Examiner O'Dell for his careful consideration of this application.

II. October 2, 2007 Interview

Applicants thank Examiner O'Dell and Examiner Desai for the October 2, 2007 interview with the undersigned, Paul Howard (Applicants' European patent counsel), David Madge and Fiona Chan-Porter (scientists at Xention, the real party in interest).

At the interview, the Examiner noted that claim 4 should have been indicated as containing allowable subject matter, and withdrew the rejection of claim 4 under 35 U.S.C. § 112, first paragraph. The Examiner also indicated that "mono-halogen", "di-halogen" and "tri-halogen" was definite in view of arguments that are made of record in this Response.

The Examiners appeared to be favorably disposed towards arguments presented at the interview, and made of record herein, regarding the commercial availability and previously documented synthesis of starting materials used to prepare the presently-claimed compounds. In addition to the availability of compounds denoted "1SM" and "2SM" by the Examiner on page 7 of the Office Action, it was also noted that compound "4SM" could have been synthesized or easily prepared by one of ordinary skill in the art. The Examiner invited applications to present these arguments in this Response.

The Examiners also appeared to be favorably disposed towards method of treatment and process claims directed towards controlling pain in view of the VR1 activity data set forth on pages 47-52 of the application as-filed (see claims 20, 23, 25 and 26). The Examiners argued, however, that claims directed to disorders besides pain were not enabled, and took the position that the "promising results" noted in the Office Action when using VR1 (also known as TRPV1) antagonists to treat COPD and asthma were not sufficient to enable the presently claimed VR1 antagonists to treat these same conditions. Applicants respectfully disagree with the Examiners' position, and request reconsideration in view of the remarks set forth in this response.

Although the Examiners appeared to concede that a skilled artisan would have no problem varying the alkylene chain length from 0 to 6 (see definition of "n" in claim 1), the Examiner asserted at the interview that the definitions of R_2 and R_3 exceed the scope of enablement provided by the application as-filed. Applicants also respectfully disagree with this contention, and request reconsideration in view of the arguments set forth below.

III. Rejections of the Claims Under 35 U.S.C. § 112, second paragraph

Claims 1-3, 5, 7, 25 and 26 stand rejected as indefinite. Applicants address these points below.

(a) The Terms "Aminocarbonyl" and "Alkoxycarbonyl" Are Definite

With respect to claims 1-3, 5 and 7, the Examiner alleges that the terms "aminocarbonyl", "alkoxycarbonyl" have no definite meaning. The term "aminocarbonyl" is nomenclature used by *Chemical Abstracts* and is synonymous with the term "carbamoyl" or $-C(O)NH_2$. (See, for example, Grant & Hackh's Chemical Dictionary, Fifth Edition (1987), attached as Exhibit A.)

The term "alkoxycarbonyl" would be understood by skilled artisans to refer to a -C(O)OR group, in which R is an alkyl group, i.e,. a carbonyl group followed by an alkoxy group. (See, for example, paragraph 33 of U.S. Published Application No. 2006/0058342, attached as Exhibit B, "the term 'alkoxycarbonyl' refers to alkyl-O-C(O)--") . This term is used in the claims of numerous patents and patent applications, including three issued patents that

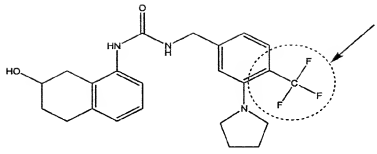
were examined by the Examiner's supervisor (See U.S. Patent Nos. 6,841,519; 6,743,814; and 6,683,107 attached as Exhibits C - E).

The Examiner asserts that carbonyl could be a portion of various groups and, as written has a dangling valance. Applicants respectfully note that definiteness of claim language must be analyzed, not in a vacuum, but in light of, *inter alia*, a) the content of the particular application disclosure and b) the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. *See* MPEP § 2173.02. A person of ordinary skill in the art would understand "amino" or "alkoxy" to refer to the substituent that follows the carbonyl group in accordance with their commonly accepted meaning (see Exhibits A and B) and cognizance that carbonyl has a valency of two and amino and alkoxy have a valency of one. Reference to the exemplified compounds, such as compound 1-24 on page 51 and compound 1-20 on page 50, which depicts an amino and alkoxy group following the carbonyl group would confirm such interpretation.

Applicants respectfully submit that a person of ordinary skill in the art would find the terms "aminocarbonyl" and "alkoxycarbonyl" to be definite when read in view of the specification as filed.

(b) The Terms "Mono-halogen", "Di-halogen" and "Tri-halogen" Are Definite

The Examiner alleged in the Office Action that the meaning of the terms "mono-halogen", "di-halogen" and "tri-halogen" is not understood. Claims 1-3 specify that R_2, R_3 and R_4 may be a C_{1-6} alkyl optionally substituted by mono-, di-, or tri-halogen. Applicants respectfully submit that such wording would be understood to signify that the C_{1-6} alkyl group may be substituted by one, two or three monovalent halogen atoms, such as the tri-fluoride substituted alkyl group shown below for compound 1-9:



(compound 1-9 set forth on page 48 of the application as filed). See also compounds 1-8, 1-10, 1-11, 1-12, 1-16, 1-17, 1-19, 1-20, 1-21, 1-22, 1-23, 1-24, 1-25, 1-26, 1-27, 1-28. Applicants respectfully submit that a person of ordinary skill in the art would ***not*** read "C_{1-6} alkyl optionally substituted by mono-, di-, or tri-halogen" to encompass compounds in which a carbon is substituted with diatomic or triatomic halogen when read in view of the specification as filed, as this would violate fundamental laws of chemistry.

The Examiner noted in the October 2, 2007 interview that the terms "mono-halogen", "di-halogen" and "tri-halogen" are definite.

(c) The Term "C_1-C_6 Alkylamino" Is Definite

The Examiner asks:

> When an optional substituent is "C_{1-6}-alkylamino" what does this encompass? Is the "C_{1-6}-alkylamino" bound to the molecule thought the N or carbon?

(Office Action, paragraph 3, pages 2-3). The Examiner is presumably referring to R_2-R_4, which may be "C_1-C_6 alkyl optionally substituted by . . . C_1-C_6 alkylamino or di(C_1-C_6 alkyl)amino."

The term "alkylamino" is described on page 13, lines 17-22 of the application:

> Alkylamino illustratively and preferably represents an alkylamino radical having one or two (independently selected) alkyl substitutents, illustratively and preferably representing methylamino, ethylamino, n-propylamino, isopropylamino, tert-butyl-amino, n-pentylamino, n-hexyl-amino, N,N-dimethylamino,

N,N-diethylamino, N-ethyl-N-methylamino, N-methyl-N-n-propylamino, N-isopropyl-N-n-propylamino, N-t-butyl-N-methylamino, N-ethyl-N-n-pentylamino and N-n-hexyl-N-methylamino.

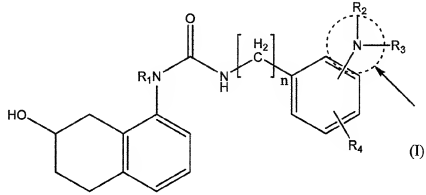
Compound 1-12 shown on page 49 of the specification as-filed demonstrates an R_2 group in which C_2 alkyl is substituted with a di(C_1alkyl)amino (i.e., N,N-dimethylamino). A person of ordinary skill in the art, when read in view of the passage above and compound 1-12, would understand the term C_{1-6}-alkylamino to refer to a -NHR group and di(C_1-C_6alkyl)amino to refer to a -NR_1R_2 group, in which R, R_1 and R_2 are a C_1-C_6 alkyl group. The C_{1-6}-alkylamino is attached to the rest of the molecule via the N atom.

(d) Formula I Clearly Indicates that "Pyrollidinyl" "Piperazinyl" "Piperidinyl" "Morpholinyl" and "Homopiperidino" are Attached Via the Nitrogen Atom

The Examiner also asks:

> When R_2 and R_3 form a "pyrollidinyl" "piperazinyl" "piperidinyl" "morpholinyl" or "homopiperidino" where is the point of attachment to the rest of the molecule?

Claim 3 recites "R_2 and R_3 ***together with the nitrogen atom to which they are attached***, form a pyrrolidinyl . . ." (emphasis added). Formula I recited in claim 1 shows the nitrogen atom attached to the aromatic ring:



(I)

Accordingly, the "pyrollidinyl", "piperazinyl", "piperidinyl", "morpholinyl" and "homopiperidinyl" moieties are attached to the rest of the molecule via the N atom.

The Examiner correctly notes that "homopiperidino" is more properly recited "homopiperidinyl". Applicants have amended claim 3 accordingly. Claim 3 has also been amended to recite "piperidinyl" instead of "piperidino". Applicants appreciate the Examiner's attention to this detail.

(e) "An Effective Amount" is Definite

In claim 25, the Examiner asserts that "a process for controlling a disorder or disease related to pain" is unclear. The Examiner also states that the wording "a VR1-antagonisticly effective amount" is improper. Claim 25 has been amended to recite "pain associated with a disease or disorder" and to recite "an effective amount".

During the October 2, 2007 interview, Examiner Desai suggested that "an effective amount" is also indefinite. Applicants respectfully disagree. A person of ordinary skill in the art could determine an effective amount of active ingredient to control pain associated with a disease or disorder via the routine titration performed by doctors in consulation with their patients. *See, e.g.,* See MPEP § 2173.05(c)III; *In re Halleck*, 422 F.2d 911 (CCPA 1970) ("an effective amount . . . for growth stimulation" deemed definite).

Furthermore, the application provides factors that one ordinary skill can consider to determine the dosage regimen:

> The dosage regimen with the use of the compounds of the present invention is selected by one of ordinary skill in the arts, in view of a variety of factors, including, without limitation, age, weight, sex, and medical condition of the recipient, the severity of the condition to be treated, the route of administration, the level of metabolic and excretory function of the recipient, the dosage form employed, the particular compound and salt thereof employed.

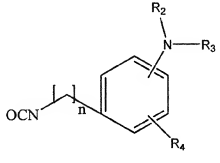
(specification as filed, page 25, lines 25-30). The application also provides examples of oral dosages (about 0.01 mg/kg/day to about 100 mg/kg/day, preferably from 0.1 mg/kg/day to 30 mg/kg/day, most preferably 0.5 mg/kg/day to about 10 mg/kg/day) and parental dosages (0.001 to 100 mg/kg/day, preferably from 0.01 mg/kg/day to 1 mg/kg/day) that could be used by the person or ordinary skill in the art as starting point (specification as filed, page 28, lines 1-9). In view of such disclosure and the stated purpose of the administration set forth in the claim, applicants submit that claim 25 is definite as amended.

IV. Rejections of the Claims Under 35 U.S.C. § 112, first paragraph

Claims 1-5, 7, 20, and 23 stand rejected as not enabled by the specification as filed. The Examiner asserts that the specifieation does not enable any person skilled in the art to make or use the invention commensurate in scope with these claims. The Examiner further alleges that only Method A is operable and asserts that Methods B through G are not operable. Applicants respectfully disagree with the Examiner's assertion regarding Methods B-G; the Examiners points are addressed below.

Method B

The Examiner states that Method B depends on the production of compound V, shown below, yet infers that a person of ordinary skill in the art would be unable to prepare such a compound:

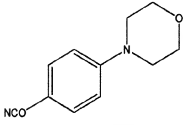


Compound V

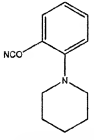
Applicants respectfully disagree with the Examiner's assertion. A person of ordinary skill in the art could either obtain or synthesize compound V.

There are numerous publications that were published prior to the filing of the present application that would allow a person of ordinary skill in the art to synthesize compound V. For example, aryl isocyantes could be prepared by a process known as the Curtius rearrangement. See, e.g., Bonjouklian, J. Org. Chemistry, 42:4095 (1977), attached as Exhibit F. A person of ordinary skill could also synthesize compound V via the Lossen rearrangment. See, e.g., Daniheer, J. Org. Chemistry, 34:2908 (1969), attached as Exhibit G.

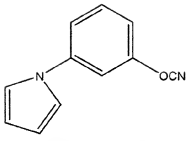
These compounds were also commercially available. Reference is made, for example, to the following compounds which were uncovered in a brief search by the Applicants:



CAS no. 884332-72-3
Commercially available from Tyger



CAS no. 892501-88-1
Commercially available from MAYBR-INT



CAS No. 857283-59-1
Available from MAYBR-INT

In view of the ability to synthesize and/or commercially obtain compound V, applicants submit that the Examiner has not established a reasonable basis to question the enablement of Method B.

Method C

The Examiner suggests that reaction of phosgene with a diamine (compound IV) and amino alcohol (compound II) would yield a urethane, and references U.S. Patent No. 3,142,699 and the publication Macromolecular Chemistry and Physics 202:3410-3419 (2001) by Bachmann et al. (hereafter "Bachmann publication").

U.S. Patent No. 3,142,699 relates to the formation of urethanes based on the reaction of semiacetals or semi-mercaptals (thioacetal) with an isocyanate. The Bachmann publication notes that phosgene may be used to transform diamino compounds into diisocynates.

Assuming that an isocyanate intermediate is formed *in situ* from napthyl amine (II), it is only under very particular conditions that a urethane would ultimately be formed. Applicants submit that the reaction would proceed as set forth on pages 17-18 of the applications under the conditions disclosed therein. In support thereof, applicants note Slocombe, J. Am Chem Soc 72: 1888 (1950), Kurita, J. Org. Chem. 41: 2070 (1976) and Cotarca, Synthesis, 553 (1996), which are attached as Exhibits H-J.

Method D

The Examiner states:

> Method D is apparently Method B, and therefore suffers from the same limitations aforementioned.

(Office Action, page 6, lines 6-7). Applicants note that Method D is similar to Method C except the isocyanate intermediate that is formed *in situ* occurs on reagent (IV). In this regard, Method D is similar to Method B. As noted with respect to Method C, the conversion of an amine to an isocyanate is well known to proceed under controlled conditions, and only yields polyurethanes under much more forced conditions.

Method E

The Examiner asserts that Method E appears to rely on the "impossible" discrimination of a chloroformate ester between two amino groups and further alleges -- without support -- that "[i]t is well known in the art that chloroformates are some of the most reactive electrophiles and they will not discriminate between these amines." (Office Action, page 6, lines 8-10)

The Examiner has not provided acceptable evidence or reasoning to support the assertion that method E requires an "impossible" discrimination between a primary alkyl amine and an aromatic amine. Those skilled in the art will appreciate that an aromatic amine is a very weakly reactive group whereas an alkyl amine is much more reactive. The Examiner has not established a reasonable basis to suggest otherwise.

In doubting the ability of a chloroformate ester to select a much more reactive alkyl amine over the less reactive aromatic amine, without providing support for such doubt, the Examiner's has not adhered to MPEP § 2164.04 which states:

> it is incumbent upon the Patent Office, whenever a rejection on [enablement] is made, to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure.

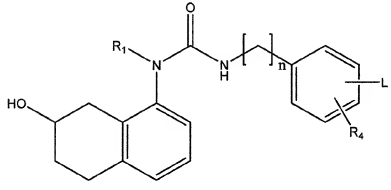
citing, *In re Marzocchi*, 439 F.2d 220, 224 (CCPA 1971). In the absence of supporting disclosure, applicants submit that the Examiner has not met the burden required to deem the reaction scheme not operable.

Method F

The Examiner states that Method F is the OH-protected version of Method B and alleges that it "suffers from the same problems as method B due to the lack of ways to make compounds such as [compound] V." Compound V however, was commercially available and/or able to be synthesized by one of ordinary skill in the art at the effective filing date of this application. Accordingly, applicants submit that Method F is an enabling scheme to prepare compounds of the present invention.

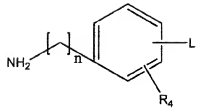
Method G

The Examiner states that "it seems possible that method G could work, although we are provided no guidance as to the source of compound X". Compound X is shown below:

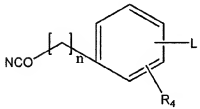


Contrary to the Examiner's assertion, however, the application as-filed provides explicit support for preparing compound X. The application states:

> The compound (X) can be prepared in a similar manner as described in Method [A], [B], [C], [D], [E], or [F] for the preparation of the compound of formula (I) using a compound (IV') or (V') (wherein n, L and R_4 are the same as defined above) instead of the compound IV or V:



Compound IV'



Compound V'

(page 23, lines 9-12, application as-filed). In view of the remarks set forth regarding methods A-F, the Examiner has not provided a reasonable basis to support a finding that Method G is not operable. To the contrary, the Examiners' own statements suggest that this method is indeed operable.

Method H

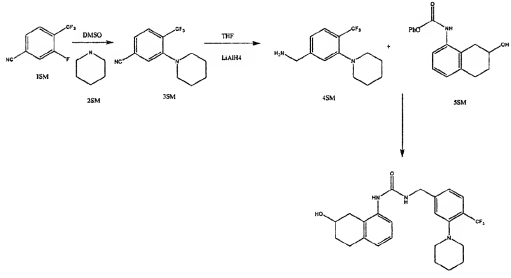
The Examiner did not address Method H, or provide any basis whatsoever to suggest that Method H is not operable. Page 23, line 13 to page 24, line 16 sets forth a detailed explanation for Method H -- *one of the eight (8) schemes that the application provides the skilled artisan to synthesize the presently claimed compounds.* Even if all of the Examiners' statements are taken as true (which Applicants strongly contest), the assertion that "the only method that is operable is method A" is incorrect since Method H is presumptively an accurate disclosure absent supporting disclosure from the Examiner that teaches otherwise. (See MPEP § 2164.04.)

Applicants note that as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. §112 is satisfied. *In re Fisher*, 427 F.2d

833, 839, 166 USPQ 18, 24 (CCPA 1970). As the Examiner has ***not*** noted any concerns with method H, applicants submit that method H alone is sufficient to find claims 1-5, 7, 20, and 23 enabled by the specification.

Method A

The Examiner reproduces the synthesis of Example 1-1 on page 7 of the Office Action:



Example 1-1

The Examiner notes "[i]n order to prepare the compounds in examples 1-2 to 1-28 we require starting materials corresponding to 1SM and 2SM or 3SM." The Examiner notes that "a quick search" of the Aldrich Chemical Company shows that only a handful of fluorobenzonitriles such as 1SM are commercially available" and sets forth 8 such commercial compounds.

(a) An Extended Period of Experimentation is Permissible Given Sufficient Guidance and Direction

Initially, applicants note that "a quick search" from only one chemical supplier is not the proper context for an enablement inquiry. MPEP § 2164.06 states:

> "[A]n extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance." (citation omitted) 'The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.'" *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (*citing In re Angstadt*, 537 F.2d 489, 502-04, 190 USPQ 214, 217-19 (CCPA 1976)).

Accordingly, "a quick search" from one supplier need not yield all the starting materials to prepare each of compounds 1-2 to 1-28, especially given the explicit reaction scheme(s) set forth in the specification, and the uncomplex starting materials (or analogs thereof) denoted by 1SM, 2SM and 3SM.

(b) Numerous Analogs of 1SM and 2SM Were Available to One of Ordinary Skill in the Art

Applicants disagree with the Examiners' assertion that only 8 analogs of 1SM are obtainable by a person of ordinary skill in the art. The Examiner asserts:

> Shockingly we cannot imagine where the trifluoromethylated compounds come from. Where can one buy or find the directions to prepare the trifluoromethylated compound 1SM to practice invention?

In response applicants note, for example, that 3-(trifluoromethyl)-4-benzonitrile (CAS No. 231953-38-1) is currently available from at least 20 suppliers shown below:

Available From:	Catalogue Number	Contact details
ABCR	AB128149	www.abcr.com
ALFA	B23791-06	www.alfa.com
APIN	46889F	www.apinchemicals.com
APOLLO	PC0891	www.apolloscientific.com

BBC-SCI	3B3-000838	www.3bsc.com
BEPAHRAM	B10794	www.bepharm.com
BETAPHARM-CD	86-17451	www.betapharma.cn
BOSCHESCI	F8559	Sales@BoscheSci.com
FLUOROCHEM	7913	www.fluorochem.net
FWDCHEM	A1735	www.fwdchem.com
INDOFINE-F	09-3587	www.indofinechemical.com
INTRCHM-FLUR	20704	www.interchim.com
JRD-FLUORO	JRD-0544	www.jrdfluoro.co.uk
MATRIX	002435	www.matrixscientific.com
MELFORD	2382	www.melford.co.uk
MOLEKULA	M2251911	www.molekula.co.uk
OAKWOOD	7913	www.oakwoodchemical.com
PIPHARM	PI-28299	www.pipharm.com
VWR	127523	www.vwr.com
WATERSTONE	17693	www.waterstonetech.com

Applicants respectfully submit that a person of ordinary skill in the art, at the time the application was filed, would have been able to obtain trifluoromethyl-benzonitrile compounds.

A quick search also revealed the following 1SM and 2SM analogs that are currently commercially available and that could be used to synthesize compounds of the present invention:

Example	Chemical name	CAS number	Supplier	Catalogue No.	Comments
I-8	3-(trifluoromethyl)-4-fluorobenzonitrile	67515-59-7	ALFA	B20256-14	1SM
I-8	3-(trifluoromethyl)-4-fluorobenzonitrile	67515-59-7	Apollo-inter	PC4373P	1SM
I-8	3-(trifluoromethyl)-4-fluorobenzonitrile	67515-59-7	fluorochem	K4583	1SM
I-8	Pyrrolidine	123-75-1	Aldrich	W352306-1KG	2SM
I-8	Pyrrolidine	123-75-1	Apollo-inter	OR0971	2SM
I-8	Pyrrolidine	123-75-1	ASDI-INTER	500010355	2SM
I-9	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM

I-9	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-9	Pyrrolidine	123-75-1	Aldrich	W352306-1KG	2SM
I-9	Pyrrolidine	123-75-1	Apollo-inter	OR0971	2SM
I-9	Pyrrolidine	123-75-1	ASDI-INTER	500010355	2SM
I-10	3-(trifluoromethyl)-4-fluorobenzonitrile	67515-59-7	ALFA	B20256-14	1SM
I-10	3-(trifluoromethyl)-4-fluorobenzonitrile	67515-59-7	Apollo-inter	PC4373P	1SM
I-10	3-(trifluoromethyl)-4-fluorobenzonitrile	67515-59-7	fluorochem	K4583	1SM
I-10	Hexamethyleneimine	111-49-9	Aldrich	H10401-100ML	2SM
I-10	Hexamethyleneimine	111-49-9	ALFA	L07906-AP	2SM
I-11	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-11	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-11	Hexamethyleneimine	111-49-9	Aldrich	H10401-100ML	2SM
I-11	Hexamethyleneimine	111-49-9	ALFA	L07906-AP	2SM
I-12	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-12	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-12	1-amino-2-dimethylaminoethane	108-00-9	Aldrich	D158003-100G	2SM
I-12	1-amino-2-dimethylaminoethane	108-00-9	ALFA	B20182-22	2SM
I-12	1-amino-2-dimethylaminoethane	108-00-9	Apollo-Inter	OR6514	2SM
I-13	2-amino-4-cyanophenol	14543-43-2	Beta Pharma	53794	
I-13	2-amino-4-cyanophenol	14543-43-2	Ramidus	1332	
I-13	4-butyrolactone	96-48-0	Aldrich	H7629-100G	
a) Ohta, Heterocycles, 1993, 36, 743-749.					
I-14	2-amino-4-cyanophenol	14543-43-2	Beta Pharma	53794	
I-14	2-amino-4-cyanophenol	14543-43-2	Ramidus	1332	
I-14	1,4-dichlorobutane	110-56-5	Aldrich	D59100-50G	
b) Hallas J Chem Soc; Perkin Trans. 2; 1984 149-154.					
I-15	1-(3-Bromo-4-fluorophenyl)methanamine	none	BetaPharma	24128	
I-15	1-(3-Bromo-4-fluorophenyl)methanamine	none	PIPharma	PI-14435	
I-15	1-(3-Bromo-4-fluorophenyl)methanamine	none	RARECHEM	AL BW 0631	
I-15	1-(3-Bromo-4-fluorophenyl)methanamine	none	UKRORGSYN-BB	BBV-025353	

I-15	piperidine	110-89-4	Aldrich	411027-100mL	2SM
I-16	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-16	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-16	Pyrrolidine	123-75-1	Aldrich	W352306-1KG	2SM
I-16	Pyrrolidine	123-75-1	Apollo-inter	OR0971	2SM
I-16	Pyrrolidine	123-75-1	ASDI-INTER	500010355	2SM
I-17	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-17	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-17	Pyrrolidine	123-75-1	Aldrich	W352306-1KG	2SM
I-17	Pyrrolidine	123-75-1	Apollo-inter	OR0971	2SM
I-17	Pyrrolidine	123-75-1	ASDI-INTER	500010355	2SM
I-19	3-(trifluoromethyl)-4-fluorobenzonitrile	67515-59-7	ALFA	B20256-14	1SM
I-19	3-(trifluoromethyl)-4-fluorobenzonitrile	67515-59-7	Apollo-inter	PC4373P	1SM
I-19	3-(trifluoromethyl)-4-fluorobenzonitrile	67515-59-7	fluorochem	K4583	1SM
I-20	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-20	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-20	4-piperidinecarboxylic acid, ethyl ester	1126-09-6	Aldrich	E33505-100G	2SM
I-20	4-piperidinecarboxylic acid, ethyl ester	1126-09-6	ALFA	B20601-22	2SM
I-20	4-piperidinecarboxylic acid, ethyl ester	1126-09-6	Apollo-Inter	OR0070	2SM
I-21	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-21	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-21	4-carboxypiperidine	498-94-2	Aldrich	I18008-100G	2SM
I-21	4-carboxypiperidine	498-94-2	ALFA	A11795-22	2SM
I-21	4-carboxypiperidine	498-94-2	Apollo-Inter	OR6923	2SM
I-22	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-22	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-22	4-(hydroxymethyl)piperidine	6457-49-4	Aldrich	497312-25G	2SM
I-22	4-(hydroxymethyl)piperidine	6457-49-4	ALFA	L17964-14	2SM

I-22	4-(hydroxymethyl)piperidine	6457-49-4	Apollo-Inter	OR3906	2SM
I-23	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-23	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-23	4-hydroxypiperidine	5382-16-1	Aldrich	128775-100G	2SM
I-23	4-hydroxypiperidine	5382-16-1	ALFA	B2273-22	2SM
I-23	4-hydroxypiperidine	5382-16-1	Apollo-Inter	OR9943	2SM
I-24	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-24	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-24	4-piperidinecarboxamide	39546-32-3	Aldrich	I17907-100G	2SM
I-24	4-piperidinecarboxamide	39546-32-3	ALFA	A13442-30	2SM
I-24	4-piperidinecarboxamide	39546-32-3	Apollo-Inter	OR28687	2SM
I-25	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-25	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-25	1-boc-piperazine	143238-38-4	Aldrich	343536-25G	2SM
I-26	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-26	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-26	Morpholine	110-91-8	Aldrich	643599-25G	2SM
I-26	Morpholine	110-91-8	ALFA	A10355-22	2SM
I-26	Morpholine	110-91-8	VWR	98346	2SM
I-27	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-27	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-27	2-(methylamino)ethanol	109-83-1	Aldrich	471445-2L	2SM
I-27	2-(methylamino)ethanol	109-83-1	ALFA	B23180-AP	2SM
I-27	2-(methylamino)ethanol	109-83-1	ASDI-INTER	500012279	2SM
I-28	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	ALFA	B23791-06	1SM
I-28	3-fluoro-4-(trifluoromethyl)benzonitrile	231953-38-1	fluorochem	7913	1SM
I-28	Morpholine	110-91-8	Aldrich	643599-25G	2SM
I-28	Morpholine	110-91-8	ALFA	A10355-22	2SM
I-28	Morpholine	110-91-8	VWR	98346	2SM

In view of the above, Applicants respectfully submit that a person of ordinary skill in the art could have obtained the starting materials 1SM and 2SM at the effective filing date of this application.

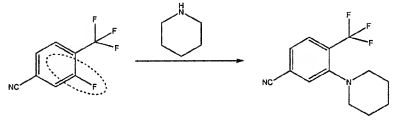
(c) The Examiner Has Not Provided a Sufficient Basis to Suggest That Nucleophilic Substitution at the Meta-Position Cannot Occur

The Examiner questions whether an activating group either in the o- or p- positions are required in order for animation to proceed, and cites *Synthesis* 2002, 2379-2382 which notes:

> Nucleophilic aromatic substitution reactions are widely used in synthetic methodology. These reactions are enhanced when the leaving group is ortho or para to an electron withdrawing species such as formyl or boronate, and in addition fluorine is a particularly good leaving group due to its substantial negative inductive effect. Nijhuls and co-workers have previously reported a similar reaction where a fluorine ortho to a formyl group was replaced by a number of N-mono-substituted piperazines.

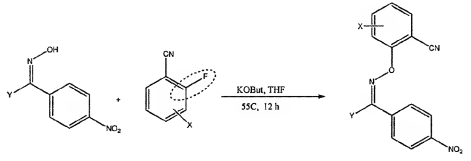
Applicants note that the *Synthesis* article does not state that it impossible for nucleophilic substitution to occur at the meta-position, only that a reaction is "enhanced" when the leaving group is ortho or para to an electron withdrawing moiety. The Examiner has not provided evidence to back up his implied assertion that meta-substituted leaving groups would not proceed to animation. See MPEP § 2164.04, *supra*. To the contrary, the passage cited by the Examiner -- which was published the same year as the effective filing date of the present application -- admits that "[n]ucleophilic aromatic substitutions reactions are widely used in synthetic methodology".

Contrary to the Examiner's position, Applicants note that the preparation of Compound D on page 44 of the application demonstrates the displacement of a fluorine group in a meta position to the site of activation:

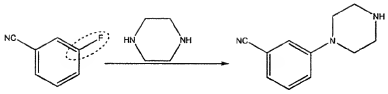


(page 44 of the application as filed) In this example, the cyano group has a stronger electron withdrawing effect than the trifluormethyl group in the ortho position to the leaving group.

Literature evidence also supports for both ortho- and meta-substitution being sufficiently activating to enable S_NAr type reactions. For example, in the case of an ortho-fluoro benzonitrile, satisfactory displacement is achieved under mild conditions:



(J. Org. Chem, 1999, 64, 4547-4550 (1999) attached as Exhibit L; see page 4547, 2nd column). Also, satisfactory displacement is shown in the literature for a meta-fluoro substituted benzonitrile:



(Tetrahedron, 55:13285-13300 (1999) attached as Exhibit M; see page 13290, scheme 4). In view of the application as-filed, which is corroborated with the literature references cited above, Applicants respectfully submit that the Examiner has not established a reasonable basis to suggest that meta-substituted (and ortho-substituted) leaving groups would not proceed to animation.

(d) The Reacting Amines are Bases and Serve as Catalysts To Convert 1SM and 2SM to 3SM

The Examiner also implies -- without justification -- that conversion of 1SM and 2SM to 3SM requires a base. In response, applicants respectfully note that the reacting amines themselves are bases; they are capable of catalyzing the reaction themselves. Also, the amines in the worked example are used in excess in comparison to the aromatic fluoride. In view of these statements, the Examiner is respectfully requested to provide a further basis for the assertion that the conversion of 1SM and 2SM to 3SM would not proceed.

(e) A Person of Ordinary Skill in the Art Could Easily Obtain Para Analogues of 1SM Via Routine Experimentation and Effort

The Examiner suggest that the para analogues of 1SM "are equally rare", yet "a simple search" performed by the Examiner yielded 7 suitable para substituted analogs of 1SM (see pages 13-14 of the Office Action). Applicants again note that every starting compound that may be used to prepare the claimed compounds need not be quickly discovered by "a simple seareh"

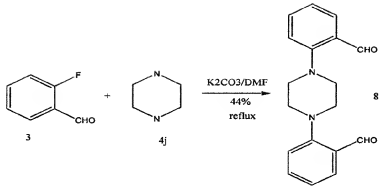
of apparently one supplier in order to satisfy the enablement requirement. *See In re Wands, supra*, which notes that a considerable amount of experimentation is permissible.

Furthermore, The Examiner admits that "the level of ordinary skill in the art is very high" as "typical researchers have a Ph.D. in organic chemistry, post-doctoral training and many years of experience in diverse fields." (Office Action, page 26, lines 7-9) Based on the relatively simple nature of the starting materials, the high level of skill in the art and the availability of at least 7 commercially available analogs that were discovered by the Examiner via "a simple search", applicants respectfully submit that the Examiner's assertions regarding a lack of para-substituted 1SM analogs does not warrant an enablement rejection. This is paricularly the case given the ready availability of the 1SM compounds discussed above in subsection (b).

(f) The Compound of Example 1-25 is Enabled; Piperazine may be Monoprotected

The Examiner alleges that some of the compounds reported -- Example 1-25 in particular -- are not enabled via the method of Example 1-1. The Examiner further states:

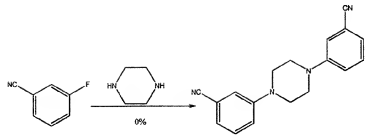
> It is well known that piperazines will of course undergo alkylation at both nitrogens see Nijhuis, et al. Synthesis 1987, 641-643, where the author states:
>
> > We have also tried to synthesize the basic skeleton 2 (R=H) in this way. Reaction of 2-fluorobenzaldehyde (3) with piperazine (4j, R=H) did not give the desired piperazinylbenzaldehyde 5j (R=H), but rather the bis-substituted piperazine 8 in 44% yield



Given the high degree of skill noted by the Examiner, it would be readily apparent to a person of ordinary skill in the art that a mono-protected piperazine could be employed to alleviate concerns regarding dual-alkylation (*See* Office Action, page 26, lines 7-9). Accordingly, applicants respectfully submit that the Examiner's concerns regarding dual-alkylation at the nitrogens are misplaced given conventional wisdom in the art. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384 (Fed. Cir. 1986) ("what is conventional or well known to one of ordinary skill in the art need not be disclosed in detail.").

Furthermore, Applicants submit that if the specification were to disclose every conceivable elementary synthesis technique, such as use of protecting groups, the result would be a voluminous specification that would render it impossible to readily ascertain the inventive subject matter of the present invention. To the contrary, the MPEP advises applicants to omit that which is well-known in the art, such as use of protecting groups. See MPEP §2164.08, *citing*, *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991) (not everything necessary to practice the invention need be disclosed. In fact, what is well-known is best omitted).

Lastly, literature suggests that it is actually difficult to achieve di-substitution of a piperazine when reacted with a substituted benzontirile compound. Exhibit M notes that the following proposed reaction had a ***zero percent yield***:



(Exhibit M, page 13290, Scheme 4). In view of the above, applicants respectfully submit that the Examiner's comments regarding dual-alkylation are misplaced.

(f) 4SM Compounds Can be Prepared Based on Ordinary Skill

The Examiner notes that claim 1 encompasses anilines and alkyl amines and asserts that the method of Example 1 cannot be used to prepare anilines corresponding to 4SM. Applicants note that the compounds set forth in Example 1 can be prepared by reacting the appropriate amine (e.g. an aniline, benzylamine or alkyl chain amine) with the same intermediate as described in Example 1-1. A person of ordinary skill in the art can select the appropriate amine and such amines are commercially available or could be readily synthesized. For example, alkyl amine intermediates can be prepared by amination of alkyl halides, and chain lengths can be modified by a homologation process. Also, simple reductive amination of a range of aldehydes could provide any number of secondary amine analogs of 8-amino-2-napthol.

(h) 4SM Compounds Were Also Commerically Available

With regard to the formation of analines and alkyl amines noted above, and also in response to the Examiner's earlier assertions regarding lack of commercially available starting materials corresponding to 1SM, it is important to note that one of ordinary skill in the art could commercially obtain starting materials corresponding to 4SM. Although it is within ordinary

skill, it is not absolutely necessary to obtain 1SM, 2SM or 3SM to prepare the presently claimed compound. These intermediates are used solely to synthesize 4SM. Starting with the first six compounds of example 1 (compounds 1-2 through 1-6) the following analogs of 4SM were readily available at the time the application was filed:

Example	Chemical name	CAS number	Supplier	Catalogue No.	Comments
I-2	1-(4-aminophenyl)piperidine	2359-60-6	Aldrich	556629-5G	4SM
I-2	1-(4-aminophenyl)piperidine	2359-60-6	ALFA	041386-06	4SM
I-2	1-(4-aminophenyl)piperidine	2359-60-6	Maybridge-int	BTB05830EB	4SM
I-2	1-(4-aminophenyl)piperidine	2359-60-6	VWR	2359-60-6	4SM
I-3	4-(4-aminophenyl)morpholine	2524-67-6	Aldrich	197157-5G	4SM
I-3	4-(4-aminophenyl)morpholine	2524-67-6	ALFA	B20292-18	4SM
I-3	4-(4-aminophenyl)morpholine	2524-67-6	Apollo-inter	OR4896	4SM
I-3	4-(4-aminophenyl)morpholine	2524-67-6	fluorochem	18189	4SM
I-3	4-(4-aminophenyl)morpholine	2524-67-6	Maybridge-Int	BTB01373EB	4SM
I-4	3-chloro-4-morpholin-4-yl-phenylamine	55048-24-3	Apollo-Inter	OR5221	4SM
I-4	3-chloro-4-morpholin-4-yl-phenylamine	55048-24-3	fluorochem	17129	4SM
I-4	3-chloro-4-morpholin-4-yl-phenylamine	55048-24-3	Maybridge-Int	DP01809EA	4SM
I-5	(4-piperidinophenyl)methylamine	214759-73-6	Apollo-Inter	OR9881	4SM
I-5	(4-piperidinophenyl)methylamine	214759-73-6	Maybridge-Int	CC33913DA	4SM
I-6	4-(4-morpholinyl)benzylamine	214759-74-7	Apollo-inter	OR23269	4SM
I-6	4-(4-morpholinyl)benzylamine	214759-74-7	ASDI-Inter	500022677	4SM
I-6	4-(4-morpholinyl)benzylamine	214759-74-7	Maybridge-Int	CC17413DA	4SM
I-7	4-(pyrrolidin-1-yl)benzylamine	114365-04-7	Apollo-inter	OR6730	4SM
I-7	4-(pyrrolidin-1-yl)benzylamine	114365-04-7	Matrix	21229	4SM
I-7	4-(pyrrolidin-1-yl)benzylamine	114365-04-7	Maybridge-Int	CC30113DA	4SM

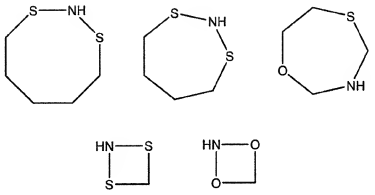
According to Chemical Abstracts, CAS numbers for 2359-60-6, 2524-67-6 and 55048-24-3 were entered into the STN database **on or before November 16, 1984** (see Exhibit P; applicants

understand that Chemical Abstracts did not track enter dates until November 16, 1984, thus they could have been entered well before 1984)

Also, art that published prior to the filing of this application would allow a person to synthesize analogs of 4SM even if they were not commercially available. N-(4-aminophenyl) piperidine (CAS Number 2359-60-6) was disclosed almost 60 years ago in the Journal of Bacteriology 56:245-52 **(1948)** (see Exhibit O). 4-morpholino aniline (CAS Number 2524-67-6) was disclosed in U.S. Patent No. 2,004,763, which issued in **1935** (see Exhibit P, column 1, line 30). Synthesis of 3-chloro-4-morpholin-4-yl phenylamine (CAS Number 55048-24-3) was disclosed in U.S. Patent No. 3,878,206, which issued in **1975** (see Exhibit Q, column 8, lines 5-18). 4-piperidin-1-ylbenzonitrile (CAS Number 1204-85-9) was disclosed in Chemische Berichte, 97(12):3268-76 **(1965)** (see Exhibit O). 4-morpholinobenzonitrile (CAS Number 10282-31-2) was disclosed in the Berichte der Bunsen-Gesellschaft 70(5):544-50 **(1966)** (see Exhibit O).

(i) Enablement Requires Only Reasonable Correlation

The Examiner alleges that claims 1 and 2 recite groups that are unknown to the Examiner, and notes the following possibilities for R_2 and R_3:



Applicants note that the language employed in claim 1 and 2 is standard language. The Examiner has used this standard language to construct theoretical molecules. This approach is not sanctioned by the MPEP or the courts, which states that the scope of enablement must only bear a "reasonable correlation" to the scope of the claims. See MPEP § 2164.08, *citing*, *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Applicants respectfully submit that the Examiner's approach of constructing hypothetical molecules demands much more than a reasonable correlation.

Furthermore, the Examiner's approach could lead to assertions that even the most elementary molecules could be deemed non-enabled. For example, a claim directed to "C_2-C_4 alkane" could be considered non-enabled under the Examiner's reasoning because it can be read to encompass C_3H_7, in which the 2nd carbon atom only has one hydrogen atom. To prevent such anomalies, MPEP § 2164.0b teaches:

> The presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled. The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art.

Applicants respectfully submit that a person of ordinary skill in the art -- whose skill is admittedly high -- could easily determine those embodiments that would be inoperative based on fundamental norms of organic chemistry.

(j) The Specification Demonstrates Utility of the Claimed Compounds

The Examiner next asserts that the specification gives "little guidance as to what these compounds do in the physiological sense." The Examiner further asserts:

> From an examination of the trends given in Table 1 it is clear that a very minor change in the structure of the antagonist results in

> dramatic changes in activity. For example a bioisoteric replacement of the 4-methlyene group of piperidine (Example 1-1) with an NH as in piperazine in Example 1-25 results in compounds with at least a 10 fold decrease in activity. The compounds of broad claims 1-3, 5-7, 25 and 27 would likely not work as antagonists.

In response, applicants note that demonstration of exceedingly beneficial results for one particular compound (e.g. Example 1-1) does not render other compounds (that still show activity) non-enabled. Applicants disagree with the assertion that a 10-fold drop in potency necessarily renders, for example, the compound of Example 1-25 non-enabled. Persons of ordinary skill in the art often sacrifice activity for other considerations, such as selectivity, physiochemical properties, etc. It may very well be that less active compounds prove, in certain particular instances applications, more helpful than compounds with higher activity.

(k) The Examiner Should Provide a Basis for Assertions Regarding Structure Modifications

The Examiner then suggest alterations to the recited compounds that, in his opinion, would prove to be more useful:

> It would appear the benzyl ring should bear a lipophilic group like trifluoromethyl or iPr. Certainly we cannot expect compounds containing sulfur or various optional substituents to function as antagonists even if they could be prepared.

Applicants respectfully request the Examiner to provide a basis for these assertions in accordance with proper enablement protocol. *See* MPEP § 2164.04 ("it is incumbent upon the Patent Office, whenever a rejection on [enablement] is made, to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure.") For example, the Examiner has provided not basis to

suggest that sulfur could not be substituted for oxygen, or that sulfur-containing drugs could not function as antagonists. *Compare,* U.S. Patent No. 5,453,426, entitled Sulfur-containing Xanthine Derivatives as Adenosine Antagonists (see Exhibit R).

(l) The Specification Demonstrates the Activity of the Claimed Compounds to Treat Pain

Finally, the Examiner notes that the application only discloses rat DRG data, and states:

> [s]o while potentially useful for treating pain in some rodents, we cannot believe that these compounds would be useful for treating pain in humans

Applicants note that IC_{50} of capsaicin-induced Ca^{2+} influx is set forth on page 40 and 47-52. At the interview, the Examiner appeared to be persuaded that such data was sufficient with respect to claims directed towards methods of treating pain.

(m) Claims Drawn to Urological Disorders are Enabled

Claims 19, 21, 22, 24, 25, and 26 stand rejected as not enabled. The Examiner asserts that claims drawn toward urological disorders are not enabled.

In the arena of pain management, it is known that both VR1 agonists (e.g. capsaicin) and VR1 antagonists both result in the reduction of VR1 activity. See, e.g., J. Biol. Chem., Vol. 280, Issue 14, 13424-13432, April 8, 2005; Cheminform, Vol. 35, Issue 12 (2004), attached as Exhibits S-T. VR1 agonists acheive reduction in VR1 activity via an initial activiation and subsequent de-sensitizing step, whereas VR1 antagonists provide an initial reduction in VR1 activity without the initial stimulatory response. As apparently accepted by the Examiner, the specification provides data in Table 1 (see pages 47-52 of the specification) analyzed under the protocol set forth in Assay 1 that demonstrates the VR1 antagonist behavior of the presently claimed compounds.

Just as with pain managment, reduction in VR1 activity is known to be useful in the treatment of urological disorders. In subjects with overactive bladder, spinal cord injury or other situations in which noxious stimulus are involved, C-fibers become active and will convey a signal ot the spinal cord resulting in a feeling of urgency to urinate or increase in the frequency

of urination. Reduction in VR1 activity, by using the VR1 agonist capsaicin, has been shown to have therapeutic utility in the treatment of neurogenic bladder dysfunction. (See C.J. Fowler, D. Jewkes, W.I. McDonald, B. Lynn and W.C. de Groat, Lancet (1992) 339 1239, Intravesical capsaicin for neurogenic bladder dysfunction, attached as Exhibit U).

It has also been shown that the TRPV1 antagonist, capsazepine, could displace the binding of radiolabelled resiniferatoxin (a natural-product TRPV1 agonist, used clinically for overactive bladder) in bladder membranes. A. Szallasi, C. Goso, P.M. Blumberg, S. Manzini, J. Pharmacol Exp. Therapeutics, (1993) 267, 728-733, Competitive inhibition by capsazepine of [3H] resiniferatoxin binding to central (spinal cord and dorsal root ganglion) and peripheral (urinary bladder and airways) vanilloid (capsaicin) receptors in the rat attached as Exhibit V

Almost immediately thereafter it was also shown that this TRPV1 antagonist (capsazepine) had a functional effect on the isolated rat bladder *ex vivo* with a demonstration of inhibition of the contractile responses to capsaicin. C.A. Maggi, S. Bevan, C.S. Walpole, H.P. Rang, S. Guiliani, British Journal of Pharmacology (1993) 108, 801-805. A comparison of capsazepine and ruthenium red as capsaicin antagonists in the rat isolated urinary bladder and vas deferens, attached as Exhibit W. This set the scene for the development of further VR1 antagonists to treat urological disorders. P. Whal, C. Foged, S. Tullinn, C. Thomsen, Molecular Pharmacology (2001) 59, 9-15, Iodo-resiniferatoxin, a new potent vanilloid receptor antagonist; Y. Wang, T. Szabo, J.D. Welter, Molecular Pharmacology (2002) 62, 947-956. High-affinity antagonists of the vanilloid receptor, attached as Exhibit X.

Given the showing of pain management in the specification and a) the known correleation between controlling pain and reducing VR1 activity and b) the known correlation between treating urological disorders and reducing VR1 activity, applicants submit that the presently claimed compounds are enabled for the treatment of urological disorders.

(m) Claims Drawn to Inflammatory Disorders (e.g. asthma and COPD) are Also Enabled

The Examiner also asserts that claims drawn to inflammatory disorders are also not enabled.

As noted above, VR1 agonists achieve an eventual reduction in VR1 activity through an initial activation and subsequent de-sensitizing step. During the activation step, capsaicin (a

TRPV1 agonist) leads to the release of neurotransmitters from both peripheral and central nerve endings, causing a collection of inflammatory responses (often referred to as neurogenic inflammation), which in turn results in bronchoconstriction, plasma extravasation and mucus hypersecretion. See H. Kanazawa, H. Fujiwara, K. Hirata, J. Yoshikawa, Eur Respir J, (1998) 12, 1307-1312, Subthreshold concentration of endothelin-1-enhanced, capsaicin-induced bronchoconstriction in anaesthetized guinea-pigs, attached as exhibit Y (bronchoconstriction); M.G. Belvisi, Curr Opin Pharmacol (2003) 3, 239-243, Airway sensory innervation as a target for novel therapies: an out-dated concept? attached as Exhibit Z (plasma extravazation); and M.G. Belvisi, Pulm Pharamcol Ther (2003) 16, 1-7. Sensory nerves and airway inflammation: role of A delta and C-fibres (mucus hypersecretion), attached as Exhibit AA.

VR1 antagonists have been shown to reduce the capsaicin-induced cough response. For example, capsazepine (a VR1 antagonist) has been been found to inhibit the cough response induced by capsaicin and citric acid. M.G. Belvisi, M. Miura, D. Stretton, P.J. Barnes, Eur J Pharmacol (1992) 215, 341-344, Capsazepine as a selective antagonist of capsaicin-induced activation of C-fibres in guinea pig bronchi, attached as Exhibit AB and U.G. Lallo, A.J. Fox, M.G. Belvisi, K.F. Chung, P.J. Barnes J Appl Physiol (1995) 79, 1082-1087, Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea pigs, attached as Exhibit AC.

Iodo-resiniferatoxin (potent TRPV1 antagonist) has also shown to inhibit capsaicin-induced cough in animals. M. Trevisani, A. Milan, R. Gatti, A. Zanasi, S. Harrison, G. Fontana, A. H. Morice, P. Geppeti, Thorax (2004) 59, 769-772, Antitussive activity of iodo-resiniferatoxin in guinea-pigs, attached as Exhibit AD.

As noted above, the VR1 antagonist effect of the presently claimed compounds is demonstrated in the application as filed. As with the VR1 antagonists capsazepine and iodo-resinferatoxin, the VR1 antagonists of the present invention would be expected to inhibit the inflammatory response that includes the inhibition of coughing, absent evdence to the contrary. Accordingly, applicants submit that the present claims are enabled based on the known function of VR1 anatagonists at the effective filing date of the present application.

V. Remarks Regarding Provisional Double-Patenting Rejections

Claims 1-3, 5, 7, 19-26 stand provisionally rejected on the ground of nonstatutory double patenting over copending application Nos. 10/513,848, 10/575,027, and 10/485,481.

Applicants note that rights to the present application and U.S.S.N. 10/513,848 was wholly assigned from Bayer Healthcare AG to Xention Limited on March 19, 2007. Applicants request that the provisional double patenting rejection of over the '848 application be held in abeyance until the finding of allowable subject matter.

Bayer Healthcare, not Xention Limited, remains the owner of U.S.S.N. 10/575,027, and 10/485,481.3. The '027, '481 and present applications are not commonly owned.

V. No Waiver

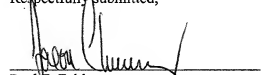
All of Applicants' arguments and amendments are without prejudice or disclaimer. Additionally, Applicants have merely discussed example distinctions from the position taken by the Examiner. Other distinctions exist, and Applicants reserve the right to discuss these additional distinctions in a later Response or on Appeal, if appropriate. By not responding to additional statements made by the Examiner, Applicants do not acquiesce to the Examiner's additional statements. The example distinctions discussed by Applicants are sufficient to overcome the Examiner's rejections.

SUMMARY

In light of the above remarks, Applicants respectfully request reconsideration and withdrawal of the outstanding rejections. Applicants further submit that the application is now in condition for allowance, and earnestly solicits timely notice of the same. Should the Examiner have any questions, comments or suggestions in furtherance of the prosecution of this application, the Examiner is invited to contact the attorney of record.

Applicants believe that there are no fees due in association with this filing of this Response, apart from the fee for filing a terminal disclaimer. However, should the Commissioner deem that any additional fees are due, including any fees for extensions of time, the Commissioner is authorized to debit Baker Botts L.L.P. Deposit Account No. 02-0383, Order Number 078503.0104, for any underpayment of fees that may be due in association with this filing.

Respectfully submitted,



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Date: October 19, 2007

EXHIBIT LIST

Exhibit A	definition of "carbamoyl" in Grant & Hach's Chemical Dictionary, Fifth Edition (1987)
Exhibit B	U.S. Published Application No. 2006/0058342
Exhibit C	U.S. Patent No. 6,841,519
Exhibit D	U.S. Patent No. 6,743,814
Exhibit E	U.S. Patent No. 6,683,107
Exhibit F	Bonjouklian, J. Org. Chemistry, 42:4095 (1977)
Exhibit G	Daniheer, J. Org. Chemistry, 34:2908 (1969)
Exhibit H	Slocombe, J. Am Chem Soc 72: 1888 (1950)
Exhibit I	Kurita, J. Org. Chem. 41: 2070 (1976)
Exhibit J	Cotarca, Synthesis, 553 (1996)
Exhibit L	J. Org. Chem, 1999, 64, 4547-4550 (1999)
Exhibit M	Tetrahedron, 55:13285-13300 (1999)
Exhibit N	Entry for CAS nos. 2359-60-6, 2524-67-6 and 55-048-24-3
Exhibit O	Abstract to Journal of Bacteriology 56:245-52 **(1948)**; Abstract to Chemische Berichte, 97(12):3268-76 **(1965)**; Abstract to Berichte der Bunsen-Gesellschaft 70(5):544-50 **(1966)** (see Exhibit U)
Exhibit P	U.S. Patent No. 2,004,763
Exhibit Q	U.S. Patent No. 3,878,206
Exhibit R	U.S. Patent No. 5,453,426
Exhibit S	J. Biol. Chem., Vol. 280, Issue 14, 13424-13432, April 8, 2005
Exhibit T	Cheminform, Vol. 35, Issue 12 (2004)
Exhibit U	C.J. Fowler, D. Jewkes, W.I. McDonald, B. Lynn and W.C. de Groat, Lancet (1992) 339 1239, Intravesical capsaicin for neurogenic bladder dysfunction
Exhibit V	A. Szallasi, C. Goso, P.M. Blumberg, S. Manzini, J. Pharmacol Exp. Therapeutics, (1993) 267, 728-733, Competitive inhibition by

	capsazepine of [3H] resiniferatoxin binding to central (spinal cord and dorsal root ganglion) and peripheral (urinary bladder and airways) vanilloid (capsaicin) receptors in the rat
Exhibit W	C.A. Maggi, S. Bevan, C.S. Walpole, H.P. Rang, S. Guiliani, British Journal of Pharmacology (1993) 108, 801-805. A comparison of capsazepine and ruthenium red as capsaicin antagonists in the rat isolated urinary bladder and vas deferens, attached as Exhibit Y
Exhibit X	Iodo-resiniferatoxin, a new potent vanilloid receptor antagonist; Y. Wang, T. Szabo, J.D. Welter, Molecular Pharmacology (2002) 62, 947-956. High-affinity antagonists of the vanilloid receptor
Exhibit Y	H. Kanazawa, H. Fujiwara, K. Hirata, J. Yoshikawa, Eur Respir J, (1998) 12, 1307-1312, Subthreshold concentration of endothelin-1-enhanced, capsaicin-induced bronchoconstriction in anaesthetized guinea-pigs
Exhibit Z	M.G. Belvisi, Curr Opin Pharmacol (2003) 3, 239-243, Airway sensory innervation as a target for novel therapies: an out-dated concept?
Exhibit AA	M.G. Belvisi, Pulm Pharamcol Ther (2003) 16, 1-7. Sensory nerves and airway inflammation: role of A delta and C-fibres
Exhibit AB	M.G. Belvisi, M. Miura, D. Stretton, P.J. Barnes, Eur J Pharmacol (1992) 215, 341-344, Capsazepine as a selective antagonist of capsaicin-induced activation of C-fibres in guinea pig bronchi
Exhibit AC	U.G. Lallo, A.J. Fox, M.G. Belvisi, K.F. Chung, P.J. Barnes J Appl Physiol (1995) 79, 1082-1087, Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea pigs
Exhibit AD	M. Trevisani, A. Milan, R. Gatti, A. Zanasi, S. Harrison, G. Fontana, A. H. Morice, P. Geppeti, Thorax (2004) 59, 769-772, Antitussive activity of iodo-resiniferatoxin in guinea-pigs

Exhibit A

GRANT & HACKH'S

CHEMICAL DICTIONARY

[*American, International, European and British Usage*]

Containing the Words Generally Used in Chemistry, and Many of the Terms Used in the Related Sciences of Physics, Medicine, Engineering, Biology, Pharmacy, Astrophysics, Agriculture, Mineralogy, etc.

Based on Recent Scientific Literature

FIFTH EDITION

Completely Revised and Edited by

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McGRAW-HILL BOOK COMPANY

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carbamide Urea. **c. chloride** Carbamoyl chloride. **c. peroxide** A solution in anhydrous glycerol, prepared from hydrogen peroxide and urea; an antiseptic.

carbamidine Guanidine*.

carbamido The ureido* radical. **phenyl ~** The phenylureido* radical.

carbaminate Carbamate*.

carbamonitrile Cyanamide.

carbamoyl* Aminocarbonyl†, carbamyl. The radical NH_2CO- from carbamic acid. **c.carbamic acid** Allophanic acid*. **c. chloride** NH_2COCl = 79.5. Carbamide chloride. Colorless needles, m.50, insoluble in water. Cf. *Friedel-Crafts reaction.*

carbamylic ester Phenylurethane.

carbanil aldehyde Formanilide.

carbanilic acid* $PhNH{\cdot}COOh$ = 123.1. Phenylcarbamic acid*. Known only as its derivatives.

carbanilide $PhNH{\cdot}CO{\cdot}NHPh$ = 212.3. Diphenylurea*. Colorless needles, m.236, slightly soluble in water.

carbanilino- The phenylcarbamoyl* radical

carbanilo- The anilino* radical. **c.nitrile** Cyanoaniline.

carbanions* Anions formed by removal of proton(s) from a C atom; e.g., benzenide, $C_6H_5^-$.

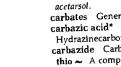
carbarsone $C_7H_9N_2O_4As$ = 260.1. *N*-Carbamoylarsanilic acid, *p*-carbaminophenylarsonic acid. White powder, insoluble in water; an antiprotozoan, used to treat amebiasis (USP). Cf. *acetarsol.*

carbates General name for the substituted dithiocarbamates.

carbazic acid* $NH_2{\cdot}NH{\cdot}COOH$ = 76.1. Hydrazinecarboxylic acid. Powder, m.ca.90 (decomp.).

carbazide Carbonohydrazide*. **semi ~** * See *semicarbazide.* **thio ~** A compound containing the $-NH{\cdot}NH{\cdot}CS{\cdot}NH{\cdot}NH-$ radical. **thiosemi ~** A compound containing the $NH_2CS{\cdot}NH{\cdot}NH-$ radical.

carbazole* $C_{12}H_9N$ = 167.2. Dibenzopyrrole, diphenylenimide.



Colorless crystals, m.245; an explosives stabilizer. **bi ~** * $C_{24}H_{16}N_2$ = 332.4. Dicarbazyl. Heterocyclic ring compounds. as 9,9'- ~ ; m. 221:

acetyl ~ See *acetylcarbazole.* ***N*-ethyl ~** $C_{14}H_{13}N$ = 196.3. Colorless crystals, m.68. **hexahydro ~** $C_{12}H_{19}N$ = 173.3. Colorless crystals, m.99. ***N*-methyl ~** $C_{13}H_{11}N$ = 182.2. Colorless crystals, m.87. **tetrahydro ~** $C_{12}H_{19}N$ = 171.2. Colorless crystals, m.119.

carbazolyl* Carbazyl. The radical $NC_{12}H_8-$ from carbazole; 5 isomers.

carbazone* A compound containing the carbazono* radical, $-N{\cdot}N{\cdot}CO{\cdot}NH{\cdot}NH-$. **thio ~** A compound containing the thicarbazono* radical, $-N{\cdot}N{\cdot}CS{\cdot}NH{\cdot}NH-$.

carbazotate Picrate*.

carbazoyl* Hydrazinocarbonyl†. The radical $NH_2{\cdot}NH{\cdot}CO-$.

carbazylic acid An organic acid of the type $RC(:NH)NH_2$

carbendazim* See *fungicides,* Table 37 on p. 250.

carbene (1)* Methylene*. The free radical $:CH_2$. (2) Cuprene.

carbenes (1) Divalent carbon compounds containing the free radical $:CX_2$. They are of low stability. (2) Constituents of bitumen, insoluble in carbon tetrachloride, soluble in carbon disulfide. Cf. *asphaltenes.*

carbenicillin (di)sodium $C_{17}H_{16}O_6N_2SNa_2$ = 422.4. Pyopen. White powder, soluble in water. An antibiotic used to treat infections due to gram-negative bacteria, particularly *Pseudomonas.*

carbenium* The cation CH_3^+.

carbeniod Describing the properties of a carbene.

carbenoxolone sodium $C_{34}H_{49}O_7Na_2$ = 614.7. Disodium enoxolone succinate. Biogastrone. White powder, m.122, slightly soluble in water. Used for peptic and mouth ulcers (BP).

carbetamide* See *herbicide,* Table 42.

carbethoxy The ethoxycarbonyl* radical.

carbethoxymino Urethane. The ethoxycarbonylimino* radical, $-NH{\cdot}CO_2Et$.

carbethylic acid Ethyl carbonate.

carbide Carbonide. carburet. Cf. *acetylide.* A binary carbon compound of a metal. With water, carbides give acetylene (Li_4C); methane (Al_4C_3); hydrogen and methane (Mg_2C_3); or a mixture of hydrogen, methane, and acetylene (rare-earth carbides). Carbides of the rare metals form solid, liquid, or gaseous hydrocarbons; some carbides (as SiC, B_4C) are extremely stable. Some contain the C_2^{--} ion. Cf. *Crystolon, Carbolay.*

carbidopa $C_{10}H_{14}O_4N_2{\cdot}H_2O$ = 244.3.White powder, slightly soluble in water. Inhibits the breakdown of dopamine in the body and thereby potentiates the effect of levodopa. q.v (USP, BP).

carbimazole $C_7H_{10}O_2N_2S$ = 186.2. Ethyl 3-methyl-2-thioxo-4-imidazoline-1-carboxylate, Neo-mercazole. White, bitter crystals, with characteristic odor, m.123, slightly soluble in water; for hyperthyroidism (BP).

carbimide Isocyanic *acid.*

carbinol Former term for (1) primary alcohols and their radical $-CH_2OH$. (2) Methanol. **acetyl ~** 1-Hydroxy-2-*propanone**. **benzyl ~** Phenethyl alcohol*. **butyl ~** **primary ~** Pentyl alcohol. **secondary ~** 2-Methyl-1-*butanol.* **tertiary ~** 2-Dimethyl*propanol**. **dimethyl ~** **secondary ~** Propanol*. **diphenyl ~** Benzohydrol. **ethyl ~** Propanol*. **methyl ~** Ethanol*. **phenyl ~** Benzyl alcohol. **propyl ~** Butanol*. **trimethyl ~** *tert*-Butyl alcohol.

Carbitol $EtO(CH_2)_2{\cdot}O{\cdot}(CH_2)_2OH$ = 134.2. Trademark for diethylene glycol ethyl ether. Colorless liquid, b.195, soluble in water; used as a solvent and in cosmetics. **butyl ~** $BuO(CH_2)_2OCH_2CH_2OH$ = 162.2. Diethylene glycol butyl ether. Colorless liquid, b.222; a solvent.

carbo Charcoal. **c. animalis** Charcoal from animal matter; decolorizing agent. **c. lignius** Wood charcoal. Used for decolorizing solutions and in blowpipe analysis. **c. sanguinarius** Blood coal. Charcoal from animal blood.

carbobenzoyl A compound containing the radical $-CO{\cdot}C_6H_4COOH$. **c.acetic acid** Colorless, m. 90. **c.formic acid** Phthalonic acid. **c.propionic acid** Colorless, m.137.

carbocinchomeronic acid Cinchomeronic acid.

carbocyanine See *cyanine dyes.*

carbocyclic* Describing a homocyclic ring compound in which all the ring atoms are carbon, e.g., benzene. Cf. *heterocyclic.*

carbodiazone* A compound containing the radical $-N{\cdot}N{\cdot}CO{\cdot}N{\cdot}N-$. **thio ~** * A compound containing the radical $-N{\cdot}N{\cdot}CS{\cdot}N{\cdot}N-$.

EXPLANATORY NOTES

Spelling. American usage is given precedence, but British forms are also listed: e.g., "sulfur" and "sulphur," respectively.

Capitals are always used in the definitions for the initial letter of the first word of a sentence.

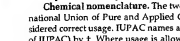
Chemical nomenclature. The two systems used internationally, namely that recommended by the International Union of Pure and Applied Chemistry (IUPAC) and that used in *Chemical Abstracts* (CA), are considered correct usage. IUPAC names are indicated by *, and CA (where different from or a less common form of IUPAC) by †. Where usage is allowed but discouraged by IUPAC (e.g., "-ic" and "-ous," as in ferric and ferrous), no * is used. See *nomenclature* for sources and other information.

Asterisks (*) are used to indicate internationally acceptable terminology or symbols. For chemical nomenclature the recommending body is IUPAC. For other fields, the main recommending body is generally as follows: SI system—CGPM; physical quantities and symbols—ISO and IUPAP; enzymes—IUB; pesticides—ISO

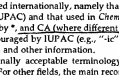
Drugs nomenclature. Entries are generally to be found under the name used by the U.S. Pharmacopoeia (USP) or National Formulary (NF). Names used in the European Pharmacopoeia (EP) and British Pharmacopoeia (BP) are cross-referenced to the USP name. However, where a compound's significance as a chemical outweighs that as a drug, the entry will be found under the chemical name. Although British and other non-American trademarks are listed under the USP or NF name, the products may not meet the USP specification.

Ring systems are represented by one of the three following methods and are numbered as shown.

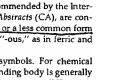
Line formula



Square formula



Geometric formula



Synonyms are given under each definition in approximate order of importance. Some of them are also listed separately in the dictionary, with a cross-reference to the entry under which the actual definition is to be found. Thus, "**brassil** Pyrite." means either (1) for "brassil" see "pyrite" or (2) same as "pyrite."

Commercial names are referred to the scientific synonym, unless the compound is of special commercial importance; e.g., "soda ash" is defined under that heading.

Italics are used only as follows: (1) according to custom (thus "*Acacia*" the plant, but "acacia" the gum; or for the letters *o-*, *m-*, *p-* used as abbreviations for *ortho*, *meta*, and *para*, respectively); (2) for cross-reference (e.g., "**acaricide** A pesticide used to kill mites. Cf. *insecticide*"); (3) for the titles of publications, etc.; (4) for certain symbols, where custom demands (see Table 88 under "symbols").

Some words may be found only as subentries of a main entry. For example, "Agent Orange" and "balancing agent" are both found only as subentries of **agent.**

For subentries such as "balancing agent" that do not begin with the same word as the main entry or that for other reasons cannot be found through a simple alphabetical search, direct cross-references take the form of "See *balancing agent* under *agent*."

When cross-references of comparison (cf.) or additional definition (q.v.) are made, only the portion of the word that occurs in the main entry is given in italics, as in the following examples: (1) "cf. balancing *agent*"; (2) "the buffer is a balancing *agent*, q.v." For the same reasons, some synonyms in the definitions may also be given partly in italics, for example, the entry for 3-phenylchromone is "**chromone** 3-phenyl ~ Iso*flavone*," indicating that "isoflavone" can be found under the main heading **flavone**.

Compound words should generally be sought under the heading of the first of the words. Thus "soda process" is to be found under "soda", and not under "process". Cross-references are sometimes given under the second word, and in some cases where importance or general custom demand, the actual definition will be found under the latter, and a cross-reference under the former.

Where the word defined is continually repeated in its main definition or subdefinition, it is represented by its initial letter followed by a period, so long as the meaning is not thereby obscured. This abbreviation can also represent the plural, where the sense so requires.

Exhibit B

US 20060058342A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2006/0058342 A1**

Firoodznia (43) **Pub. Date: Mar. 16, 2006**

(54) **IMIDAZO[1,5-A]PYRIDINE DERIVATIVES AND METHODS FOR TREATING ALDOSTERONE MEDIATED DISEASES**

(76) Inventor: **Fariborz Firoodznia**, Madison, NJ (US)

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(21) Appl. No.: **10/534,631**

(22) PCT Filed: **Nov. 17, 2003**

(86) PCT No.: **PCT/EP03/12851**

Related U.S. Application Data

(60) Provisional application No. 60/427,325, filed on Nov. 18, 2002.

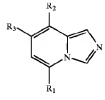
Publication Classification

(51) **Int. Cl.**
C07D 471/02 (2006.01)
A61K 31/4745 (2006.01)

(52) **U.S. Cl.** **514/303**; 546/121

(57) **ABSTRACT**

Compounds of the formula (I)

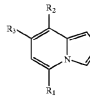


(I)

provide pharmacological agents which are inhibitors of the P450 enzyme, aldosterone syntiase, and thus may be employed for the treatment of aldosterone mediated conditions. Accordingly, the compounds of formula (I) may be employed for prevention, delay of progression, or treatment of hypokalemia, hyperiension, congestive heart failure, renal failure, in particular, chronic renal failure, restenosis, atherosclerosis, syndrome X, obesity, nephropathy, post-myocardial infarction, coronary heart diseases, increased formation of collagen, fibrosis and remodeling following hypertension and endothelial dysfunction. Preferred are the compounds of formula (I) which are selective inhibitors of aldosterone synthase devoid of undesirable side effects due to general inhibition of cytochrome P450 enzymes.

IMIDAZO[1,5-A]PYRIDINE DERIVATIVES AND METHODS FOR TREATING ALDOSTERONE MEDIATED DISEASES

[0001] The present invention provides compounds of the formula (I)



(I)

wherein

[0002] R_1 is cycloalkyl, heterocyclyl or an aryl radical of the formula

in which

[0003] R_4 is cycloalkyl, aryl or heterocyclyl; or

[0004] R_4 is optionally substituted alkyl, alkoxy, hydroxy, halogen or trifluoromethyl provided that both R_5 and R_6 are not hydrogen;

[0005] R_5 is hydrogen, halogen, cyano, alkoxy or trifluoromethyl; or

[0006] R_4 and R_5 combined together with the carbon atoms to which they are attached form an optionally substituted aromatic or heteroaromatic 5- or 6-membered ring provided that R_6 and R_3 are attached to carbon atoms adjacent to each other; or

[0007] R_4 and R_5 combined are alkylene which taken together with the carbon atoms to which they are attached form a 4- to 7-membered ring provided that R_4 and R_5 are attached to carbon atoms adjacent to each other;

[0008] R_6 is hydrogen, halogen, cyano, nitro, trifluoromethyl, optionally substituted lower alkyl, optionally substituted amino, alkoxy, carboxy, alkoxycarbonyl, sulfonyl or carbamoyl;

[0009] R_2 and R_3 are, independently, hydrogen, trifluoromethyl or alkoxy; or

[0010] R_2 and R_3 combined together with the carbon atoms to which they are attached form an optionally substituted aromatic or heteroaromatic 5- or 6-membered ring; or

[0011] R_2 and R_3 combined are alkylene which taken together with the carbon atoms to which they are attached form a 4- to 7-membered ring;

or a pharmaceutically acceptable salt thereof.

[0012] Structurally related compounds were described in the U.S. Pat. Nos. 4,588,732 and 4,617,307 respectively as thromboxane synthase inhibitors and aromatase inhibitors. The U.S. Pat. No. 6,037,349 described imidazopyridines different in its basic structure from the present compounds as having antagonistic properties towards angiotensin II.

[0013] The compounds of the present invention are inhibitors of the P450 enzyme, aldosterone synthase, and thus may be employed for the treatment of aldosterone mediated conditions. Accordingly, the compounds of formula (I) may be employed for prevention, delay of progression, or treatment of hypokalemia, hypertension, congestive heart failure, renal failure, in particular, chronic renal failure, restenosis, atherosclerosis, syndrome X, obesity, nephropathy, post-myocardial infarction, coronary heart diseases, increased formation of collagen, fibrosis and remodeling following hypertension and endothelial dysfunction. Preferred are the compounds of formula (I) which are selective inhibitors of aldosterone synthase devoid of undesirable side effects due to general inhibition of cytochrome P450 enzymes.

[0014] Listed below are definitions of various terms used to describe the compounds of the present invention. These definitions apply to the terms as they are used throughout the specification unless they are otherwise limited in specific instances either individually or as part of a larger group.

[0015] The term "optionally substituted alkyl" refers to unsubstituted or substituted straight- or branched-chain hydrocarbon groups having 1-20 carbon atoms, preferably 1-7 carbon atoms. Exemplary unsubstituted alkyl groups include methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, isobutyl, pentyl, hexyl, isohexyl, heptyl, 4,4-dimethylpentyl, octyl and the like. Substituted alkyl groups include, but are not limited to, alkyl groups substituted by one or more of the following groups: halo, hydroxy, cycloalkyl, acyl, alkoxy, alkyloxyalkoxy, alkanoyloxy, amino, alkylamino, dialkylamino, acylamino, carbamoyl, thiol, alkylthio, alkylthiono, sulfonyl, sulfonamido, sulfamoyl, nitro, cyano, carboxy, alkoxycarbonyl, alkenyl, alkynyl, aryloxy, aralkoxy, guanidino, heterocyclyl including indolyl, imidazolyl, furyl, thienyl, thiazolyl, pyrrolidyl, pyridyl, pyrimidyl, piperidyl, morpholinyl and the like.

[0016] The term "lower alkyl" refers to those optionally substituted alkyl groups as described above having 1-7, preferably 1-4 carbon atoms.

[0017] The term "halogen" or "halo" refers to fluorine, chlorine, bromine and iodine.

[0018] The term "alkenyl" refers to any of the above alkyl groups having at least two carbon atoms and further containing a carbon to carbon double bond at the point of attachment. Groups having 2-4 carbon atoms are preferred.

[0019] The term "alkynyl" refers to any of the above alkyl groups having at least two carbon atoms and further containing a carbon to carbon triple bond at the point of attachment. Groups having 2-4 carbon atoms are preferred.

[0020] The term "alkylene" refers to a straight-chain bridge of 2-5 carbon atoms connected by single bonds, e.g., $-(CH_2)_x-$, wherein x is 2-5, which may be interrupted with one or more heteroatoms selected from O, S, S(O), $S(O)_2$ or NR, wherein R may be hydrogen, alkyl, cycloalkyl,

aryl, acyl, carbamoyl, sulfonyl, sulfamoyl, alkoxycarbonyl, aryloxycarbonyl or aralkoxycarbonyl, and the alkylene may be substituted with one or more substituents selected from alkyl, cycloalkyl, oxo, halogen, hydroxy, carboxy, alkoxy, alkoxycarbonyl and the like.

[0021] The term "cycloalkyl" refers to optionally substituted monocyclic, bicyclic or tricyclic hydrocarbon groups of 3-12 carbon atoms, each of which may be substituted by one or more substituents, such as alkyl, halo, oxo, hydroxy, alkoxy, alkanoyl, acylamino, carbamoyl, alkylamino, dialkylamino, thiol, alkylthio, nitro, cyano, carboxy, alkoxycarbonyl, sulfonyl, sulfonamido, sulfamoyl, heterocyclyl and the like.

[0022] Exemplary monocyclic hydrocarbon groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl and cyclohexenyl and the like.

[0023] Exemplary bicyclic hydrocarbon groups include bornyl, indyl, hexahydroindyl, tetrahydronaphthyl, decahydronaphthyl, bicyclo[2.1.1]hexyl, bicyclo[2.2.1]heptyl, bicyclo[2.2.1]heptenyl, 6,6-dimethylbicyclo[3.1.1]heptyl, 2,6,6-trimethylbicyclo[3.1.1]heptyl, bicyclo[2.2.2]octyl and the like.

[0024] Exemplary tricyclic hydrocarbon groups include adamantyl and the like.

[0025] The term "alkoxy" refers to alkyl-O—.

[0026] The term "alkanoyl" refers to alkyl-C(O)—.

[0027] The term "alkanoyloxy" refers to alkyl-C(O)—O—.

[0028] The terms "alkylamino" and "dialkylamino" refer to alkyl-NH— and $(alkyl)_2N$—, respectively.

[0029] The term "alkanoylamino" refers to alkyl-C(O)—NH—.

[0030] The term "alkylthio" refers to alkyl-S—.

[0031] The term "alkylthiono" refers to alkyl-S(O)—.

[0032] The term "alkylsulfonyl" refers to alkyl-$S(O)_2$—.

[0033] The term "alkoxycarbonyl" refers to alkyl-O—C(O)—.

[0034] The term "alkoxycarbonyloxy" refers to alkyl-O—C(O)O—.

[0035] The term "carbamoyl" refers to $H_2NC(O)$—, alkyl-NHC(O)—, $(alkyl)_2NC(O)$—, aryl-NHC(O)—, alkyl(aryl)-NC(O)—, heteroaryl-NHC(O)—, alkyl(heteroaryl)-NC(O)—, aralkyl-NHC(O)—, alkyl(aralkyl)-N(O)— and the like.

[0036] The term "sulfamoyl" refers to $H_2NS(O)_2$—, alkyl-$NHS(O)_2$—, $(alkyl)_2NS(O)_2$—, aryl-$NHS(O)_2$—, alkyl(aryl)-$NS(O)_2$—, $(aryl)_2NS(O)_2$—, heteroaryl-$NHS(O)_2$—, aralkyl-$NHS(O)_2$—, heteroaralkyl-$NHS(O)_2$— and the like.

[0037] The term "sulfonamido" refers to alkyl-$S(O)_2$—NH—, aryl-$S(O)_2$—NH—, aralkyl-$S(O)_2$—NH—, heteroaryl-$S(O)_2$—NH—, heteroaralkyl-$S(O)_2$—NH—, alkyl-$S(O)_2$—N(alkyl)-, aryl-$S(O)_2$—N(alkyl)-, aralkyl-$S(O)_2$—N(alkyl)-, heteroaryl-$S(O)_2$—N(alkyl)-, heteroaralkyl-$S(O)_2$—N(alkyl)- and the like.

[0038] The term "sulfonyl" refers to alkylsulfonyl, arylsulfonyl, heteroarylsulfonyl, aralkylsulfonyl, heteroaralkylsulfonyl and the like.

[0039] The term "optionally substituted amino" refers to a primary or secondary amino group which may optionally be substituted by a substituent, such as acyl, sulfonyl, alkoxycarbonyl, cycloalkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, aralkoxycarbonyl, heteroaralkoxycarbonyl, carbamoyl and the like.

[0040] The term "aryl" refers to monocyclic or bicyclic aromatic hydrocarbon groups having 6-12 carbon atoms in the ring portion, such as phenyl, biphenyl, naphthyl or tetrahydronaphthyl, each of which may optionally be substituted by 1-4 substituents, such as optionally substituted alkyl, trifluoromethyl, cycloalkyl, halo, hydroxy, alkoxy, acyl, alkanoyloxy, aryloxy, optionally substituted amino, thiol, alkylthio, arylthio, nitro, cyano, carboxy, alkoxycarbonyl, carbamoyl, alkylthiono, sulfonyl, sulfonamido, heterocyclyl and the like.

[0041] The term "monocyclic aryl" refers to optionally substituted phenyl as described under aryl.

[0042] The term "aralkyl" refers to an aryl group bonded directly through an alkyl group, such as benzyl and phenethyl.

[0043] The term "aralkanoyl" refers to aralkyl-C(O)—.

[0044] The term "aralkylthio" refers to aralkyl-S—.

[0045] The term "aralkoxy" refers to an aryl group bonded directly through an alkoxy group.

[0046] The term "arylsulfonyl" refers to aryl-$S(O)_2$—.

[0047] The term "arylthio" refers to aryl-S—.

[0048] The term "aroyl" refers to aryl-C(O)—.

[0049] The term "aroyloxy" refers to aryl-C(O)—O—.

[0050] The term "aroylamino" refers to aryl-C(O)—NH—.

[0051] The term "aryloxycarbonyl" refers to aryl-O—C(O)—.

[0052] The term "heterocyclyl" or "heterocyclo" refers to an optionally substituted, fully saturated or unsaturated, aromatic or nonaromatic cyclic group, e.g., which is a 4- to 7-membered monocyclic, 7- to 12-membered bicyclic or 10- to 15-membered tricyclic ring system, which has at least one heteroatom in at least one carbon atom containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2 or 3 heteroatoms selected from nitrogen atoms, oxygen atoms and sulfur atoms, where the nitrogen and sulfur heteroatoms may also be optionally oxidized. The heterocyclic group may be attached at a carbon atom, or it may be attached through a nitrogen atom provided that an appropriately hybridized nitrogen atom constitutes part of the structure. In the case of bicyclic benzofused heterocyclic groups, the point of attachment is always at the ring containing at least one heteroatom as defined herein above.

[0053] Exemplary monocyclic heterocyclic groups include pyrrolidinyl, pyrrolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, triazolyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl,

furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, 4-piperidonyl, pyridyl, pyraziny1, pyrimidinyl, pyridazinyl, tetrahydropyranyl, morpholinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, 1,3-dioxolane and tetrahydro-1,1-dioxothienyl, 1,1,4-trioxo-1,2,5-thiadiazolidin-2-yl and the like.

[0054] Exemplary bicyclic heterocyclic groups include indolyl, dihydroindolyl, benzothiazolyl, benzoxazinyl, benzoxazolyl, benzothienyl, benzothiazinyl, quinolinyl, tetrahydroquinolinyl, decahydroquinolinyl, isoquinolinyl, tetrahydroisoquinolinyl, decahydroisoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, chromonyl, coumarinyl, benzopyranyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl, e.g., furo[2,3-c]pyridinyl, furo[3,2-b]-pyridinyl and furo[2,3-b]pyridinyl, dihydroisoindolyl, 1,3-dioxo-1,3-dihydroisoindol-2-yl, dihydroquinazolinyl, e.g., 3,4-dihydro-oxo-quinazolinyl, phthalazinyl and the like.

[0055] Exemplary tricyclic heterocyclic groups include carbazolyl, dibenzoazepinyl, dithienoazepinyl, benzindolyl, phenanthrolinyl, acridinyl, phenanthridinyl, phenoxazinyl, phenothiazinyl, xanthenyl, carbolinyl and the like.

[0056] The term "heterocyclyl" includes substituted heterocyclic groups. Substituted heterocyclic groups refer to heterocyclic groups substituted with 1, 2 or 3 substitutents selected from the group consisting of the following:

[0057] (a) alkyl;

[0058] (b) hydroxy (or protected hydroxy);

[0059] (c) halo;

[0060] (d) oxo, i.e., =O;

[0061] (e) optionally substituted amino, alkylamino or dialkylamino;

[0062] (f) alkoxy;

[0063] (g) cycloalkyl;

[0064] (h) carboxy;

[0065] (i) heterocyclooxy;

[0066] (j) alkoxycarbonyl, such as unsubstituted lower alkoxycarbonyl;

[0067] (k) mercapto;

[0068] (l) nitro;

[0069] (m) cyano;

[0070] (n) sulfamoyl or sulfonamido;

[0071] (o) aryl;

[0072] (p) alkanoyloxy;

[0073] (q) aroyloxy;

[0074] (r) arylthio;

[0075] (s) aryloxy;

[0076] (t) alkylthio;

[0077] (u) formyl;

[0078] (v) carbamoyl;

[0079] (w) aralkyl; or

[0080] (x) aryl substituted with alkyl, cycloalkyl, alkoxy, hydroxy, amino, acylamino, alkylamino, dialkylamino or halo.

[0081] The term "heterocyclooxy" denotes a heterocyclic group bonded through an oxygen bridge.

[0082] The term "heteroaryl" refers to an aromatic heterocycle, e.g., monocyclic or bicyclic aryl, such as pyrrolyl, pyrazolyl, imidazolyl, triazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, furyl, thienyl, pyridyl, pyraziny1, pyrimidinyl, pyridazinyl, indolyl, benzothiazolyl, benzoxazolyl, benzothienyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzofuryl and the like, optionally substituted by, e.g., lower alkyl, lower alkoxy or halo.

[0083] The term "heteroarylsulfonyl" refers to heteroaryl-$S(O)_2$—.

[0084] The term "heteroaroyl" refers to heteroaryl-C(O)—.

[0085] The term "heteroaroylamino" refers to heteroaryl-C(O)NH—.

[0086] The term "heteroaralkyl" refers to a heteroaryl group bonded through an alkyl group.

[0087] The term "heteroaralkanoyl" refers to heteroaralkyl-C(O)—.

[0088] The term "heteroaralkanoylamino" refers to heteroaralkyl-C(O)NH—.

[0089] The term "acyl" refers to alkanoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl and the like.

[0090] The term "acylamino" refers to alkanoylamino, aroylamino, heteroaroylamino, aralkanoylamino, heteroaralkanoylamino and the like.

[0091] Pharmaceutically acceptable salts of any compound of the present invention refer, in particular, to salts formed with acids, namely acid addition salts with the imidazolyl moiety of the structure. The acid addition salts may be formed with mineral acids, organic carboxylic acids or organic sulfonic acids, e.g., hydrochloric acid, maleic acid and methanesulfonic acid, respectively.

[0092] Similarly, salts formed with bases, e.g., cationic salts, such as alkali and alkaline earth metal salts, such as sodium, lithium, potassium, calcium, magnesium, as well as ammonium salts, such as ammonium, trimethylammonium, diethylammonium and tris(hydroxymethyl)methylammonium salts and salts with amino acids, are possible if an acidic group constitutes part of the structure.

[0093] The present invention provides bicyclic imidazole derivatives of formula (I), pharmaceutical compositions containing them, methods for preparing said compounds, and methods of treating aldosterone mediated conditions by administration of a therapeutically effective amount of a compound of the present invention or a pharmaceutical composition thereof.

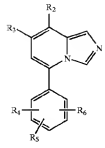
[0094] Preferred are the compounds of formula (I), wherein

[0095] R_1 is heterocyclyl;

[0096] R_2 and R_3 are hydrogen;

or pharmaceutically acceptable salt thereof.

[0097] Preferred are also the compounds of formula (I) having the formula (IA)



(IA)

wherein

[0098] R_2 and R_3 are, independently, hydrogen, trifluoromethyl or alkoxy; or

[0099] R_2 and R_3 combined together with the carbon atoms to which they are attached form an optionally substituted aromatic or heteroaromatic 5- or 6-membered ring; or

[0100] R_2 and R_3 combined are alkylene which taken together with the carbon atoms to which they are attached form a 4- to 7-membered ring;

[0101] R_4 is cycloalkyl, aryl or heterocyclyl; or

[0102] R_4 is optionally substituted alkyl, alkoxy, hydroxy, halogen or trifluoromethyl provided that both R_5 and R_6 are not hydrogen;

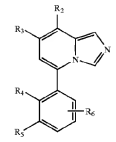
[0103] R_5 is hydrogen, halogen, cyano, alkoxy or trifluoromethyl; or

[0104] R_4 and R_5 combined together with the carbon atoms to which they are attached form an optionally substituted aromatic or heteroaromatic 5- or 6-membered ring provided that R_4 and R_5 are attached to carbon atoms adjacent to each other;

[0105] R_6 is hydrogen, halogen, cyano, nitro, trifluoromethyl, optionally substituted lower alkyl, optionally substituted amino, alkoxy, carboxy, alkoxycarbonyl, sulfonyl or carbamoyl;

or a pharmaceutically acceptable salt thereof.

[0106] Preferred are the compounds of formula (IA) having the formula (IB)



(IB)

wherein

[0107] R_2 and R_3 are, independently, hydrogen, trifluoromethyl or alkoxy; or

[0108] R_2 and R_4 combined together with the carbon atoms to which they are attached form aromatic or heteroaromatic 5- to 6-membered ring;

[0109] R_4 is cycloalkyl, aryl or heterocyclyl; or

[0110] R_4 is hydroxy, halogen or trifluoromethyl provided that both R_5 and R_6 are not hydrogen;

[0111] R_5 is hydrogen, halogen, cyano, alkoxy or trifluoromethyl; or

[0112] R_4 and R_5 combined together with the carbon atoms to which they are attached form an optionally substituted aromatic or heteroaromatic 5- or 6-membered ring;

[0113] R_6 is hydrogen, halogen, cyano, nitro, trifluoromethyl, optionally substituted lower alkyl, optionally substituted amino, alkoxy, carboxy, alkoxycarbonyl, sulfonyl or carbamoyl;

or a pharmaceutically acceptable salt thereof.

[0114] Preferred are compounds of formula (IB), wherein

[0115] R_2 and R_3 are hydrogen;

or a pharmaceutically acceptable salt thereof.

[0116] Preferred are also compounds of formula (IB), wherein

[0117] R_4 is monocyclic aryl or heteroaryl;

[0118] R_5 is hydrogen;

[0119] R_6 is hydrogen, halogen, cyano, trifluoromethyl or alkoxy;

or a pharmaceutically acceptable salt thereof.

[0120] Preferred are also compounds of formula (IB), wherein

[0121] R_4 and R_5 combined together with the carbon atoms to which they are attached form an optionally substituted aromatic or heteroaromatic 5- or 6-membered ring;

[0122] R_6 is hydrogen, halogen, cyano, trifluoromethyl or alkoxy;

or a pharmaceutically acceptable salt thereof.

[0123] Particular embodiments of the invention are:

[0124] 5-Naphthalen-1-yl-imidazo[1,5-a]pyridine;

[0125] 5-Biphenyl-4-yl-imidazo[1,5-a]pyridine;

[0126] 5-Biphenyl-2-yl-imidazo[1,5-a]pyridine;

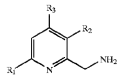
[0127] 5-Benzofuran-3-yl-imidazo[1,5-a]pyridine; and

[0128] 4-Imidazo[1,5-a]pyridin-5-yl-3,6-dihydro-2H-pyridine-1-carboxylic acid benzyl ester;

or a pharmaceutically acceptable salt thereof.

[0129] The compounds of the invention depending on the nature of the substituents, may possess one or more asymmetric centers. The resulting diastereoisomers, enantiomers and geometric isomers, and mixtures thereof, are encompassed by the instant invention.

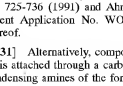
[0130] Compounds of formula (I), wherein R_1, R_2 and R_3 have meanings as defined herein above, may be prepared from amines of the formula (II)



(II)

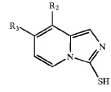
wherein R_1, R_2 and R_3 have meanings as defined for formula (I), using methods well-known in the art, e.g., according to methods described by Browne et al., *J. Med. Chem.*, Vol. 34, pp. 725-736 (1991) and Ahmad et al., International PCT Patent Application No. WO 01/27107, or modifications thereof.

[0131] Alternatively, compounds of formula (I), wherein R_1 is attached through a carbon atom, may be prepared by condensing amines of the formula (III)



(III)

wherein R_2 and R_3 have meanings as defined herein above, with an isothiocyanate, such as phenylisothiocyanate, in an organic solvent, such as toluene or xylenes to afford thiols of the formula (IV)



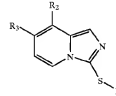
(IV)

wherein R_2 and R_3 have meanings as defined for formula (III). Amines of formula (III) are known, or if they are novel, they may be prepared according to methods well-known in the art, e.g., as described by Browne et al., supra and Ahmad et al., supra, or modifications thereof.

[0132] Thiols of formula (IV), wherein R_2 and R_3 have meanings as defined herein above, may then be treated with an alkylating agent of the formula (V)

$$R_7—Lg_1 \quad (V)$$

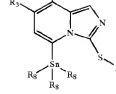
wherein R_7 represents an alkyl group, preferably ethyl; and Lg_1 is a leaving group, such as chloride, bromide or Iodide, preferably iodide; to afford thioethers of the formula (VI)



(VI)

wherein R_2 and R_3 have meanings as defined for formula (IV); and R_7 has a meaning as defined for formula (V); in the presence of a base, such as potassium or cesium carbonate and an organic solvent, such as acetone or acetonitrile, respectively. The alkylation reaction may be conducted at a temperature ranging from room temperature (RT) to a temperature near the boiling point of the solvent.

[0133] Thioethers of formula (VI), wherein R_2, R_3 and R_7 have meanings as defined herein above, may be converted to tin derivatives of the formula (VII)



(VII)

wherein R_4, R_5 and R_7 have meanings as defined for formula (VI); and R_8 represents lower alkyl, such as methyl, ethyl or n-butyl, preferably n-butyl, by treating thioethers of formula

(VI) with a base, such as n-butyllithium to form an anion, and then reacting the resulting anion with a tin reagent of the formula (VIII)

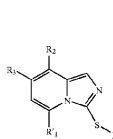
(VIII)

wherein R_8 has a meaning as defined for formula (VII); and Lg_2 represents a leaving group, such as chloride or bromide, preferably chloride. The reaction may be conducted in an inert organic solvent, such as pentane, hexane, tetrahydrofuran (THF) or ethyl ether, or in a mixture of solvents thereof, at a temperature ranging from about −45° C. to about −100° C. Preferably, the reaction is conducted in THF at a temperature of about −78° C.

[0134] Compounds of formula (VII), wherein R_2, R_3, R_7 and R_8 have meanings as defined herein above, may be coupled with a compound of the formula (IX)

(IX)

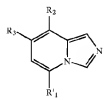
wherein R^{1a} represents R_1 as defined herein above; or R_1' is a group convertible to R_1; Lg_3 is a leaving group, such as chloride, bromide, iodide or trifluoromethanesulfonate; and R_1' is attached to Lg_3 through a carbon atom, under conditions of Stille coupling to afford compounds of the formula (X)



(X)

wherein R_2, R_3 and R_7 have meanings as defined for formula (VII); and R_1' is as defined for formula (IX). For example, compounds of formula (X) may be obtained by reacting compounds of formula (VII) with a compound of formula (IX) in the presence of a palladium(0) catalyst such as tris(dibenzylidineacetone)dipalladium(0) and a ligand, such as tri-t-butylphosphine or triphenylarsine in an organic solvent, such as THF, 1,4-dioxane, N,N-dimethylformamide (DMF) or N-methylpyrrolidine (NMP). The coupling reaction may be carried out in the presence of an additive such as lithium chloride or cesium fluoride at a temperature ranging from RT to a temperature near the boiling point of the solvent.

[0135] Compounds of formula (X), wherein R_1', R_2, R_3 and R_7 have meanings as defined herein above, may be converted to compounds of the formula (I')



(I')

wherein R_1', R_2 and R_3 have meanings as defined for formula (X), by desulfurization, e.g., in the presence of Raney nickel in a polar organic solvent, such as a lower alcohol. Preferably, the lower alcohol is ethanol, and the reaction is conducted at a temperature near the boiling point of the solvent.

[0136] The processes described herein above may be conducted under inert atmosphere, preferably under nitrogen atmosphere.

[0137] In starting compounds and intermediates which are converted to the compounds of the invention in a manner described herein, functional groups present, such as amino, thiol, carboxyl and hydroxy groups, are optionally protected by conventional protecting groups that are common in preparative organic chemistry. Protected amino, thiol, carboxyl and hydroxyl groups are those that can be converted under mild conditions into free amino thiol, carboxyl and hydroxyl groups without the molecular framework being destroyed or other undesired side reactions taking place.

[0138] The purpose of introducing protecting groups is to protect the functional groups from undesired reactions with reaction components under the conditions used for carrying out a desired chemical transformation. The need and choice of protecting groups for a particular reaction is known to those skilled in the art and depends on the nature of the functional group to be protected (hydroxyl group, amino group, etc.), the structure and stability of the molecule of which the substituent is a part and the reaction conditions.

[0139] Well-known protecting groups that meet these conditions and their introduction and removal are described, e.g., in McOmie, "*Protective Groups in Organic Chemistry*", Plenum Press, London, New York (1973); and Greene and Wuts, "*Protective Groups in Organic Synthesis*", John Wiley and Sons, Inc., New York (1999).

[0140] The above-mentioned reactions are carried out according to standard methods, in the presence or absence of diluent, preferably, such as are inert to the reagents and are solvents thereof, of catalysts, condensing or said other agents, respectively and/or inert atmospheres, at low temperatures, RT or elevated temperatures, preferably at or near the boiling point of the solvents used, and at atmospheric or super-atmospheric pressure. The preferred solvents, catalysts and reaction conditions are set forth in the appended illustrative Examples.

[0141] The invention further includes any variant of the present processes, in which an intermediate product obtain-

able at any stage thereof is used as starting material and the remaining steps are carried out, or in which the starting materials are formed in situ under the reaction conditions, or in which the reaction components are used in the form of their salts or optically pure antipodes.

[0142] Compounds of the invention and intermediates can also be converted into each other according to methods generally known per se.

[0143] The invention also relates to any novel starting materials, intermediates and processes for their manufacture.

[0144] Depending on the choice of starting materials and methods, the new compounds may be in the form of one of the possible isomers or mixtures thereof, for example, as substantially pure geometric (cis or trans) isomers, diastereomers, optical isomers (antipodes), racemates or mixtures thereof. The aforesaid possible isomers or mixtures thereof are within the purview of this invention.

[0145] Any resulting mixtures of isomers can be separated on the basis of the physicochemical differences of the constituents, into the pure geometric or optical isomers, diastereomers, racemates, for example, by chromatography and/or fractional crystallization.

[0146] Any resulting racemates of final products or intermediates can be resolved into the optical antipodes by known methods, e.g., by separation of the diastereomeric salts thereof, obtained with an optically active acid or base, and liberating the optically active acidic or basic compound. In particular, the imidazolyl moiety may thus be employed to resolve the compounds of the present invention into their optical antipodes, e.g., by fractional crystallization of a salt formed with an optically active acid, e.g., tartaric acid, dibenzoyl tartaric acid, diacetyl tartaric acid, di-O,O'-p-toluoyl tartaric acid, mandelic acid, malic acid or camphor-10-sulfonic acid. Racemic products can also be resolved by chiral chromatography, e.g., high pressure liquid chromatography (HPLC) using a chiral adsorbent.

[0147] Finally, compounds of the invention are either obtained in the free form, as a salt thereof, or as prodrug derivatives thereof.

[0148] Compounds of the invention may be converted into acid addition salts thereof, in particular, acid addition salts with the imidazolyl moiety of the structure, preferably pharmaceutically acceptable salts thereof. These are formed, e.g., with inorganic acids, such as mineral acids, e.g., sulfuric acid, a phosphoric or hydrohalic acid, or with organic carboxylic acids, such as (C_1-C_4)alkanecarboxylic acids which, for example, are unsubstituted or substituted by halogen, e.g., acetic acid, such as saturated or unsaturated dicarboxylic acids, e.g., oxalic, succinic, malelc or fumaric acid, such as hydroxycarboxylic acids, e.g., glycolic, lactic, malic, tartaric or citric acid, such as amino acids, e.g., aspartic or glutamic acid, or with organic sulfonic acids, such as (C_1-C_4)alkylsulfonic acids, e.g., methanesulfonic acid; or arylsulfonic acids which are unsubstituted or substituted, e.g., by halogen. Preferred are salts formed with hydrochloric acid, methanesulfonic acid and maleic acid.

[0149] Compounds of the instant invention, provided an acidic group constitutes part of the structure, may be converted into salts with pharmaceutically acceptable bases. Such salts include alkali metal salts, like sodium, lithium and potassium salts; alkaline earth metal salts, like calcium and magnesium salts; ammonium salts with organic bases, e.g., trimethylamine salts, diethylamine salts, tris(hydroxymethyl)methylamine salts, dicyclohexylamine salts and N-methyl-D-glucamine salts; salts with amino acids like arginine, lysine and the like. Salts may be formed using conventional methods, advantageously in the presence of an ethereal or alcoholic solvent, such as a lower alkanol. From the solutions of the latter, the salts may be precipitated with ethers, e.g., diethyl ether. Resulting salts may be converted into the free compounds by treatment with acids. These or other salts can also be used for purification of the compounds obtained.

[0150] Prodrug derivatives of any compound of the invention are derivatives of said compounds which following administration release the parent compound in vivo via some chemical or physiological process, e.g., a prodrug on being brought to the physiological pH or through enzyme action is converted to the parent compound. Exemplary prodrug derivatives are, e.g., esters of free carboxylic acids and S-acyl and O-acyl derivatives of thiols, alcohols or phenols, wherein acyl has a meaning as defined herein. Preferred are pharmaceutically acceptable ester derivatives convertible by solvolysis under physiological conditions to the parent carboxylic acid, e.g., lower alkyl esters, cycloalkyl esters, lower alkenyl esters, benzyl esters, mono- or di-substituted lower alkyl esters, such as the ω-(amino, mono- or di-lower alkylamino, carboxy, lower alkoxycarbonyl)-lower alkyl esters, the α-(lower alkanoyloxy, lower alkoxycarbonyl or di-lower alkylaminocarbonyl)-lower alkyl esters, such as the pivaloyloxymethyl ester and the like conventionally used in the art.

[0151] In view of the close relationship between the free compounds, the prodrug derivatives and the compounds in the form of their salts, whenever a compound is referred to in this context, a prodrug derivative and a corresponding salt is also intended, provided such is possible or appropriate under the circumstances.

[0152] The compounds, including their salts, can also be obtained in the form of their hydrates, or include other solvents used for their crystallization.

[0153] The pharmaceutical compositions according to the invention are those suitable for enteral, such as oral or rectal, transdermal and parenteral administration to mammals, including man, to inhibit aldosterone synthase, and for the treatment of conditions associated with aldosterone synthase activity. Such conditions include hypokalemia, hypertension, congestive heart failure, renal failure, in particular chronic renal failure, restenosis, atherosclerosis, syndrome X, obesity, nephropathy, post-myocardial infarction, coronary heart diseases, increased formation of collagen, fibrosis and remodeling following hypertension and endothelial dysfunction. The said pharmaceutical compositions comprise a therapeutically effective amount of a pharmacologically active compound of the instant invention, alone or in combination with one or more pharmaceutically acceptable carriers.

[0154] The pharmacologically active compounds of the invention are useful in the manufacture of pharmaceutical compositions comprising a therapeutically effective amount thereof in conjunction or admixture with excipients or

carriers suitable for either enteral or parenteral application. Preferred are tablets and gelatin capsules comprising the active ingredient together with diluents, e.g., lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine; lubricants, e.g., silica, talcum, stearic acid, its magnesium or calcium salt and/or polyethyleneglycol; for tablets also binders, e.g., magnesium aluminum silicate, starch paste, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone; if desired disintegrants, e.g., starches, agar, alginic acid or its sodium salt, or effervescent mixtures; and/or absorbants, colorants, flavors and sweeteners.

[0155] Injectable compositions are preferably aqueous isotonic solutions or suspensions, and suppositories are advantageously prepared from fatty emulsions or suspensions. Said compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or buffers. In addition, they may also contain other therapeutically valuable substances. Said compositions are prepared according to conventional mixing, granulating or coating methods, respectively, and contain about 0.1-75%, preferably about 1-50%, of the active ingredient.

[0156] Suitable formulations for transdermal application include a therapeutically effective amount of a compound of the invention with carrier. Advantageous carriers include absorbable pharmacologically acceptable solvents to assist passage through the skin of the host. Characteristically, transdermal devices are in the form of a bandage comprising a backing member, a reservoir containing the compound optionally with carriers, optionally a rate controlling barrier to deliver the compound of the skin of the host at a controlled and pre-determined rate over a prolonged period of time, and means to secure the device to the skin.

[0157] The pharmaceutical formulations contain a therapeutically effective amount of a compound of the invention as defined above, either alone or in a combination with another therapeutic agent, e.g., each at an effective therapeutic dose as reported in the art. Such therapeutic agents include anti-obesity agents, such as orlistat, anti-hypertensive agents, inotropic agents and hypolipidemic agents, e.g., loop diuretics, such as ethacrynic acid, furosemide and torsemide; angiotensin converting enzyme (ACE) inhibitors, such as benazepril, captopril, enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril and trandolapril; inhibitors of the Na-K-ATPase membrane pump, such as digoxin; neutralendopeptidase (NEP) inhibitors; ACE/NEP inhibitors, such as omapatrilat, sampatrilat and fasidotril; angiotensin II antagonists, such as candesartan, eprosartan, irbesartan, losartan, telmisartan and valsartan, in particular, valsartan; β-adrenergic receptor blockers, such as acebutolol, atenolol, betaxolol, bisoprolol, metoprolol, nadolol, propranolol, sotalol and timolol; inotropic agents, such as digoxin, dobutamine and milrinone; calcium channel blockers, such as amlodipine, bepridil, diltiazem, felodipine, nicardipine, nimodipine, nifedipine, nisoldipine and verapamil; and 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoA) inhibitors, such as lovastatin, pitavastatin, simvastatin, pravastatin, cerivastatin, mevastatin, velostatin, fluvastatin, dalvastatin, atorvastatin, rosuvastatin and rivastatin. A compound of the present invention may be administered either simultaneously, before or after the other active ingredient, either separately by the same or different route of administration or together in the same pharmaceutical formulation.

[0158] The structure of the active agents identified by code nos., generic or trade names may be taken from the actual edition of the standard compendium "The Merck Index" or from databases, e.g. Patents International (e.g. IMS World Publications). The corresponding content thereof is hereby incorporated by reference.

[0159] Thus in an additional aspect the present invention concerns a pharmaceutical composition comprising a therapeutically effective amount of a compound of the present invention in combination with one or more pharmaceutically acceptable carriers.

[0160] In a further aspect the present invention relates to a combination, such as a combined preparation or pharmaceutical composition, respectively, comprising a compound of the invention and another therapeutic agent as described above preferably an anti-obesity agent, anti-hypertensive agent, inotropic agent or hypolipidemic agent.

[0161] For instance, the present invention concerns a pharmaceutical composition comprising a therapeutically effective amount of a compound of the invention in combination with a therapeutically effective amount of another therapeutic agent as described above preferably an anti-obesity agent, anti-hypertensive agent, inotropic agent or hypolipidemic agent.

[0162] The invention concerns furthermore;

[0163] A pharmaceutical composition or combination as described above for use as a medicament.

[0164] The use of a pharmaceutical composition or combination as described above for the preparation of a medicament for the treatment of conditions associated with aldesterone synthase activity, preferably hypokalemia, hypertension, congestive heart failure, renal failure, in particular, chronic renal failure, restenosis, atherosclerosis, syndrome X, obesity, nephropathy, post-myocardial infarction, coronary heart diseases, increased formation of collagen, fibrosis and remodeling following hypertension and endothelial dysfunction.

[0165] A pharmaceutical composition, e.g. for use in any of the conditions described above comprising a compound of the invention in free form or pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent or carrier therefore.

[0166] A pharmaceutical composition as described above for the treatment of conditions associated with aldosterone synthase activity, preferably hypokalemia, hypertension, congestive heart failure, renal failure, in particular, chronic renal failure, restenosis, atherosclerosis, syndrome X, obesity, nephropathy, post-myocardial infarction, coronary heart diseases, increased formation of collagen, fibrosis and remodeling following hypertension and endothelial dysfunction.

[0167] A unit dosage for a mammal of about 50-70 kg may contain between about 1-1000 mg, advantageously between about 5-500 mg of the active ingredient. The therapeutically effective dosage of a compound of formula (I) is dependent

on the species of warm-blooded animal (mammal), the body weight, age and individual condition, on the form of administration and on the compound involved.

[0168] The compounds of the present invention are inhibitors of aldosterone synthase, and thus may be employed for the treatment of conditions associated with aldosterone synthase activity, as described herein, e.g., hypokalemia, hypertension, congestive heart failure, renal failure, in particular, chronic renal failure, restenosis, atherosclerosis, syndrome X, obesity, nephropathy, post-myocardial infarction, coronary heart diseases, increased formation of collagen, fibrosis and remodeling following hypertension and endothelial dysfunction. Thus, in an additional embodiment, the present invention relates to;

[0169] A compound of the present invention for use as a medicament.

[0170] The use of a compound of the invention for the preparation of a pharmaceutical composition for the prevention and/or treatment of conditions associated with aldosterone synthase activity.

[0171] A method for the prevention and/or treatment of conditions associated with with aldosterone synthase activity, which comprises administering a therapeutically effective amount of a compound of the present invention.

[0172] In accordance with the foregoing the present invention provides in a yet further aspect:

[0173] A therapeutic combination, e.g. a kit, kit of parts e.g. for use in any method as defined herein, comprising a compound of the invention, in free form or in pharmaceutically acceptable salt form, to be used concomitantly or in sequence with at least one pharmaceutical composition comprising at least another therapeutic agent as described above, preferably an anti-obesity agent, anti-hypertensive agent, inotropic agent or hypolipidemic agent. The kit may comprise instructions for its administration.

[0174] A kit of parts comprising

[0175] (i) a pharmaceutical composition of the invention, (ii) a pharmaceutical composition comprising a compound selected from an another therapeutic agent as described above preferably an anti-obesity agent, anti-hypertensive agent, inotropic agent or hypolipidemic agent, or a pharmaceutically acceptable salt thereof,

[0176] in the form of two separate units of the components (i) to (ii).

[0177] A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of a compound of the invention in free form or In pharmaceutically acceptable salt form, and a second therapeutic agent, said second therapeutic agent being selected from the other therapeutic agents described above preferably an anti-obesity agent, anti-hypertensive agent, inotropic agent or hypolipidemic agent.

[0178] Preferably the compound of the invention is administered to a mammal in need thereof.

[0179] Preferably the compound of the invention is used for the treatment of a disease which responds to activation of conditions associated with aldosterone synthase activity.

[0180] Preferably the conditions associated with aldosterone synthase activity are selected from hypokalemia, hypertension, congestive heart failure, renal failure, in particular, chronic renal failure, restenosis, atherosclerosis, syndrome X, obesity, nephropathy, post-myocardial infarction, coronary heart diseases, increased formation of collagen, fibrosis and remodeling following hypertension and endothelial dysfunction.

[0181] A method or use according to the invention which comprises administering said compound in combination with a therapeutically effective amount of another therapeutic agent as described above preferably an anti-obesity agent, anti-hypertensive agent, inotropic agent or hypolipidemic agent.

[0182] Preferably the invention relates to; i) a use or method for the treatment of hypokalemia, hypertension, congestive heart failure, atherosclerosis, coronary heart diseases and post myocardial infarction, which method comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of the invention; ii) a use or method for the treatment of restenosis, increased formation of collagen, fibrosis, and remodeling following hypertension and endothelial dysfunction, which method comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of the invention; iii) a use or a method for the treatment of renal failure and nephropathy, which method comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of the invention; iv) a use or a method for the treatment of syndrome X and obesity, which method comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of the invention.

[0183] A method or use according to the invention which comprises administering said compound in the form of a pharmaceutical composition or combination as described herein.

[0184] As used throughout the specification and in the claims, the term "treatment" embraces all the different forms or modes of treatment as known to those of the pertinent art and in particular includes preventive, curative, delay of progression and palliative treatment.

[0185] The above-cited properties are demonstrable in vitro and in vivo tests using advantageously mammals, e.g., mice, rats, dogs, monkeys or isolated organs, tissues and preparations thereof. Said compounds can be applied in vitro in the form of solutions, e.g., preferably aqueous solutions, and in vivo either enterally, parenterally, advantageously intravenously, e.g., as a suspension or in aqueous solution. The dosage in vitro may range between about 10^{-3} molar and 10^{-9} molar concentrations. A therapeutically effective amount in vivo may range depending on the route of administration, between about 0.1-500 mg/kg, preferably between about 1-100 mg/kg.

[0186] The activity of a compound according to the invention can be assessed by the following methods or methods well-described in the art:

[0187] The aldosterone synthase inhibitory activity in vitro may be determined as follows:

[0188] Adult male Sprague-Dawley rats weighing 125-150 g are obtained from Harlan Farms. All animals are caged in pairs and maintained under standard conditions of light and temperature. The animals are placed on a sodium depleted diet (0.01-0.02%) from Harland Teklad, Madison Wis. (Cat. No. TD90228) and maintained on normal drinking water ad libitum. The animals are maintained on this diet for two to four weeks prior to harvesting the adrenal glomerulosa cells. The rats are killed by CO_2 inhalation, and the adrenals are immediately removed and placed in the same ice-cold buffer used during homogenization and assay of the enzyme preparation. The adrenals are de-capsulated to obtain the glomerulosa tissue. The tissue is homogenized in a glass homogenizer containing Tris assay buffer (8.5 mM $MgCl_2$, 2.7 mM $CaCl_2$, 3.13 mM KCl, 7.59 mM NaCl, 0.1% TEA and 50 mM Tris HCl adjusted to pH 7.4). The homogenate is diluted so that 37.5 mg of glomerulosa tissue is in each mL of buffer. The homogenate is centrifuged at 4° C. at 900xg for 10 min. To start the assay, and 200 μl. aliquots (450-550 μg of protein) of the adrenal cytosolic preparation is added to each glass tube containing 2.5×10^{-6} M NADPH, 4×10^{-5} M corticosterone. The final corticosterone concentration consisted of 4×10^{-6} M corticosterone (C-2505, Sigma Chemical Co, St. Louis, Mo.) and 1×10^{-8} M [1,2,6, 7-^{3}H] corticosterone (70 CVmM; NET 399; NEN™ Life Sciences Product, Inc., Boston, Mass.) and various concentrations of the putative aldosterone synthase inhibitor as indicated. The final volume of the incubation mixture is 0.5 mL. The mixture is incubated for 1 h at 25+ C. in a Dubnoff shaking incubator at 1 atm of 95% O_2/5% CO_2. The reaction is stopped by the addition of 7 mL EtOAc, and the steroids extracted after vortexing. The water phase is extracted again with 3 mL of EtOAc. The combined extracts are dried under nitrogen, reconstituted in EtOAc, and spotted on silica gel TLC plates (LK6F; Cat. No. 4866-820; Whatman, Inc. Clifton, N.J.). The chromatograms are developed in a solvent system of toluene:acetone:water (120:80:0.8 %/v) for 60 min. The plates are scanned for radioactivity with a Biosean System 200 Imaging Scanner (Bioscan, Ish. DC). The two products of the aldosterone synthase, 18-OH-corticosterone and aldosterone are scraped and counted in a liquid scintillation counter (Beckman LS6000TA, Beckman. Instr., Palo Alto, Calif.).

[0189] The IC_{50} values are determined from a logit-log plot (pseudo-Hill plot) according to the equation (see Pratt and Taylor, Eds, *"Principles of Drug Action"*, Churchill Livingstone Inc, New York (1990)):

$$\log P/(100-P) = n \log[I] + n \log IC_{50}$$

wherein P is the percent competition of specific binding in the presence of a given concentration of inhibitor (I). The slope (Hill Coefficient) and x intercept (IC_{50}) are determined by linear regression of the experimental data. The Km(app) is calculated by a computer program according to the Hanes equation (see Cornish-Bowden, Ed., *"Principles of Enzyme Kinetics"*, Butterworth & Co., Boston, Mass. (1976)):

$$s/v = Km/V + s/N$$

wherein Km=Michaelis constant, V=maximum velocity, s=substrate concentration, v=velocity.

[0190] The aromatase inhibitory activity in vitro may be determined as follows:

[0191] Human placental microsome fraction is prepared from freshly delivered human term placenta as previously described with minor modifications (see Steele et al., *Steroids*, Vol. 50, pp. 147-161 (1987)). The tissue is freed of membranes and large vessels and rinsed repeatedly with 0.15 M KCl (4° C.). It is then minced in 0.25 M sucrose and homogenized. The homogenate is centrifuged at 20,000xg for 30 min. The supernatant is then centrifuged at 148,000xg for 60 min. The microsomal pellet obtained is re-suspended in 0.05 M potassium phosphate buffer pH 7.4 and centrifuged again at 148,000xg for 60 min. The resulting pellet is re-suspended in phosphate buffer, divided into aliquots, and stored at −40° C.

[0192] Human placental aromatase assay is performed in a incubation mixture consisting of: 12.5 mM phosphate buffer (12.5 mM KH_2PO_4, 1 mM EDTA, 1.6 mM dithiothreitol and 1.0 g/L of albumin; pH 7.5), NADPH (2.4×10^{-4} M), 1β-^{3}H androstenedione (1×10^{-7} M) and the appropriate concentration of the desired inhibitor. The assay is started by pipetting the appropriate amount 50-500 μg of the human placental microsome preparation into the incubation mixture. The mixture is incubated at 37° C. for 20 min and stopped by addition of 6 volumes of chloroform. The samples are immediately vortexed and centrifuged. The aqueous layer is carefully removed so as to avoid contamination with chloroform. The aqueous fraction is treated with an equal volume of a 5% aqueous suspension of charcoal to remove any substrate not extracted by the chloroform. After centrifugation an aliquot of the aqueous phase is counted in a liquid scintillation counter. The enzymatic activity for each concentration of inhibitor is calculated as a percent of the vehicle control, which is arbitrarily set at 100%. Therefore, the relative enzyme inhibition is expressed as a percentage: 100% minus % enzyme activity with inhibitor present.

[0193] The aldosterone synthase inhibitory activity for reduction of cardiac damage in vivo may be evaluated as follows:

[0194] The protocol is nearly identical to that previously described (see Rocha et al., *Endocrinology*, Vol. 141, pp. 3871-3878 (2000)) with minor modifications. The rats are housed in individual cages and given 0.9% NaCl as drinking fluid ad libitum throughout the experiment. Three days later rats are placed on one of the three dosing protocols. Group 1 (control) receives L-NAME for 14 days, and on day 11 of L-NAME treatment, an osmotic mini-pump containing only saline is implanted in each animal subcutaneously (s.c.). Group 2 (L-NAME/Ang II) received L-NAME for 14 days, and on day 11 of L-NAME treatment, an osmotic mini-pump containing Ang II is implanted in each animal s.c. Group 3 (L-NAME/Ang II/test compound) is treated similarly to Group 2 but receives test compound (4 mg/kg/day) orally once a day. The test compound is dissolved in distilled water and given by gavage; whereas Groups 1 and 2 receive the vehicle. The experiment is concluded on day 14 of L-NAME treatment. The L-NAME (Sigma Chemical Co., St. Louis, Mo.) is administered in the 0.9% NaCl drinking water at a concentration of 60 mg/100 mL which results in a daily

intake of approximately 60 mg/kg. Ang II is administered via Alzet osmotic mini-pumps (Model 2001; Alza Corp, Palo Alto, Calif.). The mini-pump is implanted s.c. at the nape of the neck. Ang II (human, 99% peptide purity) is purchased from Sigma Chemical Co., St. Louis, Mo. and administered at a dose of 225 μg/kg/day in saline. The concentration of Ang II used to fill the pumps is calculated based upon: (a) the mean pump rate provided by the manufacturer; (b) the body weight of the animals on the day before implantation of the pumps; and (c) dose planned.

[0195] The rats are sacrificed on day 14. Their hearts are removed and sliced through the ventricle/atrium in a "bread-loaf" manner, yielding three samples from the following gross cardiac regions: superior, middle and inferior. The samples are fixed in 10% buffered formalin. Paraffin sections are cut and stained with hematoxylin/eosin. A single investigator who is blinded to the experimental groups views slides. One slide from each of three gross cardiac sample regions is analyzed per rat. Cardiac sites (left and right ventricles and the septum) are evaluated separately. The entire section is assessed histologically for the presence of myocardial damage (regardless of the severity) as evidenced by the presence of myocyte necrosis, inflammatory cells, hemorrhage and general tissue disruption. Evaluation of the hisiological data is made by comparing Groups 2 and 3, i.e., ANG II with or without test compound.

[0196] Illustrative of the invention, the compound of Example 1 inhibits the aldosterone synthase activity with an IC_{50} value of about 50 nM.

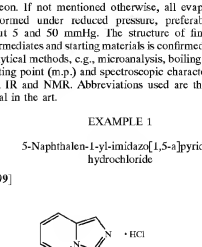
[0197] Our compounds exhibit high affinity, selectivity, improved potency as well as good oral bioavailability, pharmacokinetic profile and safety. Furthermore, they exhibit long duration of action, long-term tolerability.

[0198] The following Examples are intended to illustrate the invention and are not to be construed as being limitations thereon. If not mentioned otherwise, all evaporations are performed under reduced pressure, preferably between about 5 and 50 mmHg. The structure of final products, intermediates and starting materials is confirmed by standard analytical methods, e.g., microanalysis, boiling point (b.p.), melting point (m.p.) and spectroscopic characteristics, e.g., MS, IR and NMR. Abbreviations used are those conventional in the art.

EXAMPLE 1

5-Naphthalen-1-yl-imidazo[1,5-a]pyridine hydrochloride

[0199]



[0200] A. Imidazo[1,5-a]pyridine-3-thiol

[0201] Phenyl Isothiocyanate (22 mL, 0.184 mol) is added slowly to a solution of 2-(aminomethyl)-pyridine (19 mL, 0.184 mol) in xylenes (180 mL) with cooling on ice. The resulting solution is heated at reflux for 3 h, and then allowed to cool to RT overnight. The resulting suspension is cooled to 0° C. and the solid is collected by filtration. The solid is washed with toluene and ether, and dried under vacuum to yield imidazo[1,5-a]pyridine-3-thiol as a green solid: ^{1}H-NMR ($CDCl_3$) δ 12.9 (br s, 1H), 8.25 (dd, J=7.5, 0.9, 1H), 7.21 (d, J=9.3, 1H), 7.06 (s, 1H), 6.75 (dd, J=9.3, 6.3, 1H), 6.59 (dt, J=7.5, 0.9); MS (M+1)$^+$151.

[0202] B. 3-Ethylsulfanyl-imidazo[1,5-a]pyridine

[0203] A 250 mL round-bottom flask is charged with acetone (92 mL), the title A compound, imidazo[1,5-a] pyridine-3-thiol (21.26 g, 0.141 mol), potassium carbonate (39.14 g, 0.283 mol) and ethyl iodide (12.4 mL, 0.155 mol). The resulting suspension is heated at 45° C. for 12 h. The reaction is cooled to RT and filtered. The solid is washed with acetone and the filtrate is concentrated under reduced pressure. The residue is dissolved in methylene chloride and filtered. The filtrate is concentrated under reduced pressure and the residue is distilled under vacuum through a 6 cm Vigreux column to yield 3-ethylsulfanyl-imidazo[1-5a]pyridine (b.p. 168-172 at 2.1 mmHg) as a yellow oil: ^{1}H-NMR ($CDCl_3$) δ 8.18 (dd, J=6.6, 0.6, 1H), 7.52 (s, 1H), 7.43 (d, J=9.0, 1H), 6.76 (dd, J=9.0, 6.3 1H), 6.64 (app. t, J=6.3, 1H), 2.95 (q, J=7.2, 2H), 1.25 (t, J=7.2, 3H); MS (M+1)$^+$179.

[0204] C. 3-Ethylsulfanyl-5-tributylstannyl-imidazo[1,5-a]pyridine

[0205] n-Butyllithium (1.6 M, 4.2 mL, 6.7 mmol) is added slowly to a solution of the title B compound, 3-ethylsulfanyl-5-methyl-imidazo[1,5-a]pyridine (1.14 g, 6.4 mmol) in anhydrous THF (7 mL) at −78° C. The resulting solution is stirred at −78° C. for 1 h and tributyltinchloride (2.0 mL, 7.4 mmol) is added. The reaction is stirred at −78° C. for 1 h further, and then allowed slowly to warm to RT overnight. The reaction is quenched by addition of saturated aqueous ammonium chloride (15 mL) and the mixture is extracted two times with ethyl acetate. The organic extracts are combined, washed with aqueous potassium fluoride solution (3 M, 20 mL), dried over anhydrous magnesium sulfate, filtered and concentrated. The residue is purified by flash column chromatography on silica using 10% ethyl acetate in hexanes as the eluant to yield 3-ethylsulfanyl-5-tributylstannyl-imidazo[1,5-a]pyridine as a dark oil: ^{1}H-NMR ($CDCl_3$) δ 7.51 (s, 1H), 7.38 (dd, J=8.7, 1.2, 1H), 6.71 (dd, J=6.3, 1.5, 1H), 6.63 (dd, J=8.7, 6.3, 1H), 2.98 (q, J=7.4, 2H), 1.58-1.48 (m, 6H), 1.40-1.17 (m, 15H), 0.88 (t, J=7.5, 9H); ^{13}C-NMR ($CDCl_3$) δ 140.27, 133.53, 132.20, 124.88, 124.76, 124.63, 121.89, 118.77, 118.65, 118.56, 32.22, 29.66, 29.37, 29.30, 29.22, 27.94, 27.79, 27.68, 27.42, 15.59, 15.52, 14.83, 14.12, 14.03, 12.71, 12.65; MS (M+1)$^+$465, 466, 467, 468, 469 (tin isotope pattern).

[0206] D. 3-Ethylsulfanyl-5-naphthalen-1-yl-imidazo[1,5-a]pyridine

[0207] To a solution of the title C compound, 3-ethylsulfanyl-5-tributylstannyl-imidazo-[1,5-a]pyridine (550 mg, 1.18 mmol) in 1,4-dioxane (8 mL), 1-bromonaphthalene (0.18 mL, 1.29 mmol) is added followed by tris(dibenzylidineacetone)dipalladium(0) (38 mg, 0.041 mmol), tri-t-butylphosphine (80 μL, 0.329 mmol) and cesium fluoride (396 mg, 2.6 mmol). The suspension is heated at 100° C. under nitrogen in a sealed tube for two days. The mixture is cooled, diluted with ethyl ether and filtered through a short silica plug. The solvent is evaporated under reduced pressure, and the residue is purified by flash column chromatography on silica using 10% ethyl acetate in hexanes to yield 3-ethylsulfanyl-5-naphthalen-1-yl-imidazo[1,5-a]pyridine as an amber oil: ^{1}H-NMR ($CDCl_3$) δ 8.01 (dd, J=7.5, 1.8, 1H), 7.93 (d, J=8.1, 1H), 7.61 (s, 1H), 7.58-7.47 (m, 4H), 7.41-7.32 (m, 2H), 6.78 (dd, J=9.0, 6.6, 1H), 6.51 (dd, J=6.6, 1.2, 1H), 2.66 (q, J=7.2, 2H), 0.86 (t, J=7.2, 3H); MS (M+1)+305.

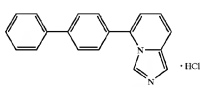
[0208] E. 5-Naphthalen-1-yl-imidazo[1,5-a]pyridine hydrochloride

[0209] Raney Nickel suspension (1 mL, Fluka Cat. No. 83440) is added to a solution of the title D compound, 3-ethylsulfanyl-5-naphthalen-1-yl-imidazo[1,5-a]pyridine (268 mg, 0.88 mmol) in ethanol (15 mL) and heated at reflux overnight. Additional Raney Nickel (1 mL) is added, and the reaction mixture is heated at reflux for three days further, until MS showed complete disappearance of the starting material. The catalyst is filtered off and washed with ethanol. The combined filtrates are concentrated under reduced pressure. The residue is purified by flash column chromatography on silica using ethyl ether as the eluant to yield 5-naphthalen-1-yl-imidazo[1,5-a]pyridine as an amber film. The hydrochloride salt is prepared in acetone by addition of an ethereal solution of HCl (1.2 eq.). The resulting precipitate is re-crystallized from acetonitrile to yield 5naphthalen-1-yl-imidazo[1,5-a]pyridine hydrochloride (m.p. 222-223° C.): ^{1}H-NMR, (MeOH-d_4) δ 8.71 (s, 1H), 8.21 (d, J=8.1, 1H), 8.16 (s, 1H), 8.13 (d, J=16.3, 1H), 8.04 (d, J=17, 1H), 7.98-7.70 (m, 2H), 7.61 (dt, J=5.6, 1.2, 1H); 7.53-7.40, m, (3H); 7.28 (d, J=7.7, 1H); ^{13}C-NMR (MeOH-d_4) δ 135.8, 135.53, 132.84, 132.68, 131.96, 130.34, 130.13, 129.81, 129.11, 128.15, 127.04, 126.18, 125.60, 124.79, 120.93, 119.43, 116.50, 112.96; MS (M+1)+245.

EXAMPLE 2

5-Biphenyl-4-yl-imidazo[1,5-a]pyridine hydrochloride

[0210]

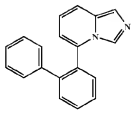


[0211] The title compound is prepared analogously to Example 1 (m.p. 240-242° C.): ^{1}H-NMR (MeOH-d_4) δ 9.43 (s,1H), 8.14 (d, J=1.5, 1H), 7.93-7.82 (m, 5H), 7.74-7.71 (m, 2H), 7.53-7.47 (m, 2H), 7.44-7.37 (m, 2H), 7.19 (dd, J=6.0, 0.9, 1H); ^{1} ^{3}C-NMR (MeOH-d_4) δ 145.11, 141.09, 137.29, 132.94, 131.80, 130.29, 130.19, 129.32, 128.16, 126.36, 125.42, 119.26, 118.84, 112.72; MS (M+1)+271.

EXAMPLE3

5-Biphenyl-2-yl-imidazo[1,5-a]pyridine hydrochloride

[0212]

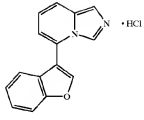


[0213] The title compound is prepared analogously to Example 1 (m.p. 200-201 ° C.): ^{1}H-NMR (MeOH-d_4) δ 8.75 (s, 1H), 7.86 (d, J=1.3, 1H), 7.79-7.35 (m, 2H), 7.69-7.62 (m, 3H), 7.33 (dd, J=6.8, 2.4, 1H), 7.26-7.21 (m, 3H); 7.17-7.12 (m, 3H); ^{13}C-NMR (MeOH-d_4) δ 142.64, 140.92, 137.14, 132.71, 132.32, 132.23, 131.95, 131.18, 129.94, 129.58, 129.26, 129.14, 129.04, 125.91, 125.70, 121.22, 118.73, 112.49; MS (M+1)+271.

EXAMPLE 4

5-Benzofuran-3-yl-imidazo[1,5-a]pyridine hydrochloride

[0214]

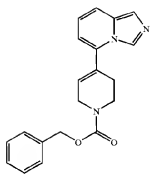


[0215] The title compound is prepared analogously to Example 1 (m.p. 96-98° C.): ^{1}H-NMR (MeOH-d_4) δ 9.40 (s, 1H), 8.48 (s, 1H), 8.15 (s, 1H), 7.93 (dd, J=9.0, 1H), 7.72 (d, J=6.0, 1H), 7.58 (d, J=9.0, 1H), 7.50 (t, J=6.0, 1H), 7.41 (m, 3H); MS (M+1)+234.

EXAMPLE 5

4-Imidazo[1,5-a]pyridin-5-yl-3,6-dihydro-2H-pyridine-1-carboxylic acid benzyl ester hydrochloride

[0216]

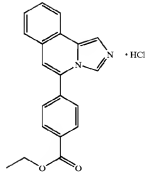


[0217] The title compound is prepared analogously to Example 1 (m.p. 149-152° C.): ^{1}H-NMR (MeOH-d_4) δ 8.08 (s, 1H), 7.80 (d, J=7.3, 1H), 7.39-7.29 (m, 5H), 7.05 (d, J=5.8, 1H), 6.41 (s, 1H), 5.19 (s, 2H), 4.28 (s, 2H), 3.83 (s, 2H), 2.60 (s, 2H); MS (M+1)+333.

EXAMPLE 6

4-Imidazo[5,1-a]isoquinolin-5-yl-benzoic acid ethyl ester hydrochloride

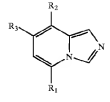
[0218]



[0219] The title compound is prepared analogously to Example 1: ^{1}H-NMR ($CDCl_3$) δ 8.24 (d, J=8.1, 2H), 8.07 (d, J=6.4, 2H), 7.93 (s, 1H), 7.77 (d, J=8.1, 2H), 7.63 (d, J=7.7, 1H), 7.56 (t, J=7.4, 1H), 7.46(t, J=7.3, 1H), 6.8 (s, 1H), 4.45(q, J=7.1, 2H), 1.45 (t,J=7.1, 3H); MS (M+1)+316.

1-21. (canceled)

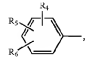
22. A compound of the formula (I)



(I)

wherein

R_1 is cycloalkyl, heterocyclyl or an aryl radical of the formula



in which

R_4 is cycloalkyl, aryl or heterocyclyl; or

R_4 is optionally substituted alkyl, alkoxy, hydroxy, halogen or trifluoromethyl provided that both R_5 and R_6 are not hydrogen;

R_5 is hydrogen, halogen, cyano, alkoxy or trifluoromethyl; or

R_4 and R_5 combined together with the carbon atoms to which they are attached form an optionally substituted aromatic or heteroaromatic 5- or 6-membered ring provided that R_4 and R_5 are attached to carbon atoms adjacent to each other; or

R_4 and R_6 combined are alkylene which taken together with the carbon atoms to which they are attached form a 4- to 7-membered ring provided that R_4 and R_6 are attached to carbon atoms adjacent to each other;

R_6 is hydrogen, halogen, cyano, nitro, trifluoromethyl, optionally substituted lower alkyl, optionally substituted amino, alkoxy, carboxy, alkoxycarbonyl, sulfonyl or carbamoyl;

R_2 and R_3 are, independently, hydrogen, trifluoromethyl or alkoxy; or

R_2 and R_3 combined together with the carbon atoms to which they are attached form an optionally substituted aromatic or heteroaromatic 5- or 6-membered ring; or

R_2 and R_3 combined are alkylene which taken together with the carbon atoms to which they are attached form a 4- to 7-membered ring;

or a pharmaceutically acceptable salt thereof.

23. A compound according to claim 22, wherein

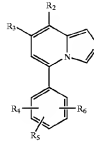
R_1 is heterocyclyl;

R_2 and R_3 are hydrogen;

or pharmaceutically acceptable salt thereof.

24. A compound according to claim 22 of the formula (IA)

(IA)



wherein

R_2 and R_3 are, independently, hydrogen, trifluoromethyl or alkoxy; or

R_2 and R_3 combined together with the carbon atoms to which they are attached form an optionally substituted aromatic or heteroaromatic 5- or 6-membered ring; or

R_2 and R_3 combined are alkylene which taken together with the carbon atoms to which they are attached form a 4- to 7-membered ring;

R_4 is cycloalkyl, aryl or heterocyclyl; or

R_4 is optionally substituted alkyl, alkoxy, hydroxy, halogen or trifluoromethyl provided that both R_5 and R_6 are not hydrogen;

R_5 is hydrogen, halogen, cyano, alkoxy or trifluoromethyl; or

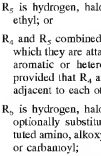
R_4 and R_5 combined together with the carbon atoms to which they are attached form an optionally substituted aromatic or heteroaromatic 5- or 6-membered ring provided that R_4 and R_5 are attached to carbon atoms adjacent to each other;

R_6 is hydrogen, halogen, cyano, nitro, trifluoromethyl, optionally substituted lower alkyl, optionally substituted amino, alkoxy, carboxy, alkoxycarbonyl, sulfonyl or carbamoyl;

or a pharmaceutically acceptable salt thereof.

25. A compound according to claim 24 of the formula (IB)

(IB)



wherein

R_2 and R_3 are, independently, hydrogen, trifluoromethyl or alkoxy; or

R_2 and R_3 combined together with the carbon atoms to which they are attached form aromatic or heteroaromatic 5- to 6-membered ring;

R_4 is cycloalkyl, aryl or heterocyclyl; or

R_4 is hydroxy, halogen or trifluoromethyl provided that both R_5 and R_6 are not hydrogen;

R_5 is hydrogen, halogen, cyano, alkoxy or trifluoromethyl; or

R_4 and R_5 combined together with the carbon atoms to which they are attached form an optionally substituted aromatic or heteroaromatic 5- or 6-membered ring;

R_6 is hydrogen, halogen, cyano, nitro, trifluoromethyl, optionally substituted lower alkyl, optionally substituted amino, alkoxy, carboxy, alkoxycarbonyl, sulfonyl or carbamoyl;

or a pharmaceutically acceptable salt thereof.

26. A compound according to claim 25, wherein

R_2 and R_3 are hydrogen;

or a pharmaceutically acceptable salt thereof.

27. A compound according to claim 25, wherein

R_4 is monocyclic aryl or heteroaryl;

R_5 is hydrogen;

R_6 is hydrogen, halogen, cyano, trifluoromethyl or alkoxy;

or a pharmaceutically acceptable salt thereof.

28. A compound according to claim 25, wherein

R_4 and R_5 combined together with the carbon atoms to which they are attached form an optionally substituted aromatic or heteroaromatic 5- or 6-membered ring;

R_6 is hydrogen, halogen, cyano, trifluoromethyl or alkoxy;

or a pharmaceutically acceptable salt thereof.

29. A compound according to claim 22 which is selected from:

5-Naphthalen-1-yl-imidazo[1,5-a]pyridine;

5-Biphenyl-4-yl-imidazo[1,5-a]pyridine;

5-Biphenyl-2-yl-imidazo[1,5-a]pyridine;

5-Benzofuran-3-yl-imidazo[1,5-a]pyridine; and

4-Imidazo[1,5-a]pyridin-5-yl-3,6-dihydro-2H-pyridine-1-carboxylic acid benzyl ester;

or a pharmaceutically acceptable salt thereof.

30. A method for the inhibition of aldosterone synthase activity in mammals which method comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of claim 22.

31. A method for the prevention and/or treatment of conditions associated with aldosterone synthase activity in mammals which method comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of claim 22.

32. A method according to claim 31, which method comprises administering said compound in combination with a therapeutically effective amount of anti-obesity agent, anti-hypertensive agent, inotropic agent or hypolipidemic agent.

33. A method for the prevention and/or treatment of conditions associated with aldosterone synthase activity in mammals which method comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of claim 25.

34. A method for the treatment of hypokalemia, hypertension, congestive heart failure, renal failure, in particular, chronic renal failure, restenosis, atherosclerosis, syndrome X, obesity, nephropathy, post-myocardial infarction, coronary heart diseases, increased formation of collagen, fibrosis and remodeling following hypertension and endothelial dysfunction, which method comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of claim 22.

35. A pharmaceutical composition comprising a therapeutically effective amount of a compound of claim 22 in combination with one or more pharmaceutically acceptable carriers.

36. A pharmaceutical composition according to claim 35 for the treatment of hypokalemia, hypertension, congestive heart failure, atherosclerosis, coronary heart diseases, post myocardial infarction, restenosis, increased formation of collagen, fibrosis, and remodeling following hypertension, endothelial dysfunction, renal failure, nephropathy, syndrome X and obesity.

37. A pharmaceutical composition comprising a therapeutically effective amount of a compound of claim 22 in combination with a therapeutically effective amount of anti-obesity agent, anti-hypertensive agent, inotropic agent or hypolipidemic agent.

38. A pharmaceutical composition according to claim 37 for the treatment of hypokalemia, hypertension, congestive heart failure, atherosclerosis, coronary heart diseases, post myocardial infarction, restenosis, increased formation of collagen, fibrosis, and remodeling following hypertension, endothelial dysfunction, renal failure, nephropathy, syndrome X and obesity.

39. A combination, comprising a compound of claim 22 and another therapeutic agent selected from an anti-obesity agent, anti-hypertensive agent, inotropic agent or hypolipidemic agent.

* * * * *

Exhibit C

US006841519B1

(12) **United States Patent**
Nakatani et al.

(10) **Patent No.: US 6,841,519 B1**
(45) **Date of Patent: Jan. 11, 2005**

(54) **ISOXAZOLINE DERIVATIVES AND HERBICIDES CONTAINING THE SAME AS THE ACTIVE INGREDIENT**

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(73) Assignees: **Kumiai Chemical Industry Co., Ltd.**, Tokyo (JP); **Ihara Chemical Industry Co., Ltd.**, Tokyo (JP)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/049,039**

(22) PCT Filed: **Aug. 9, 2000**

(86) PCT No.: **PCT/JP00/05325**

§ 371 (c)(1),
(2), (4) Date: **Feb. 8, 2002**

(87) PCT Pub. No.: **WO01/12613**

PCT Pub. Date: **Feb. 22, 2001**

(30) **Foreign Application Priority Data**

Aug. 10, 1999 (*JP*) 11-225878

(51) **Int. Cl.**[7] **A01N 43/80**; C07D 261/04

(52) **U.S. Cl.** **504/271**; 548/243; 514/378

(58) **Field of Search** 504/271; 548/243; 514/378

(56) **References Cited**

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Kazuo Tomita et al.: "Studies on Isoxazoles. IX. Synthesis of 3–Phenoxy–, 3–Phenylthio– and 3–Alkylthioisoxazoles", Chemical & Pharmaceutical Bulletin, vol. 27, No. 10, pp. 215–2423, (1979).

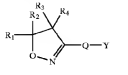
* cited by examiner

Primary Examiner—Rita Desai
Assistant Examiner—Ebenezer Sackey
(74) *Attorney, Agent, or Firm*—Kubovcik & Kubovcik

(57) **ABSTRACT**

The isoxazoline derivative of the present invention is represented by the following general formula [I]:

[I]



[wherein Q is a group represented by $—S(O)_n—(CR_5R_6)_m-$ (wherein n is an integer of 0 to 2, m is an integer of 1 to 3, and R_5 and R_6 are a hydrogen atom, a cyano group, an alkoxycarbonyl group, if an alkyl group or the like); R_1 and R_2 are a hydrogen atom, all optionally substituted alkyl group, an alkoxycarbonyl group, an optionally substituted phenyl group or the like; R_3 and R_4 are a hydrogen atom, an optionally substituted alkyl group, a cycloalkyl group or the like; Y is a hydrogen atom, an alkoxycarbonyl group, a carboxyl group, an alkenyl group, an optionally substituted alkyl group or an optionally substituted phenyl group]. The herbicide of the present invention contains the above isoxazoline derivative as the active ingredient.

27 Claims, No Drawings

ISOXAZOLINE DERIVATIVES AND HERBICIDES CONTAINING THE SAME AS THE ACTIVE INGREDIENT

This application is a 371 of PCT/JP00/05325 filed Aug. 9, 2000.

TECHNICAL FIELD

The present invention relates to a novel isoxazoline derivative and a herbicide containing it as the active ingredient.

BACKGROUND ART

The herbicidal activities of isoxazoline derivatives are reported in, for example, JP-A-8-225548, JP-A-9-328477 and JP-A-9-328483. The compounds described in these literatures have a chloromethyl group mainly at the 5-position of the isoxazoline ring, and the isoxazoline derivative of the present invention has been unknown.

Herbicides used for useful crops are desired to be applied to soil or foliage, show a sufficient herbicidal effect at a low amount, and exhibit high selectivity between crop and weeds. The compounds described in the above literatures are not fully satisfactory in these respects.

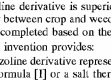
DISCLOSURE OF THE INVENTION

In view of the above situation, the present inventors made a study on herbicidal effect and selectivity between crop and weeds. As a result, the present inventors found out that a novel isoxazoline derivative is superior in herbicidal effect and selectivity between crop and weeds. The present invention has been completed based on the finding.

The present invention provides:

(1) an isoxazoline derivative represented by the following general formula [I] or a salt thereof:

[I]



(wherein Q is a group represented by —S(O)$_n$—(CR$_5$R$_6$)$_m$— (wherein n is an integer of 0 to 2, m is an integer of 1 to 3, and R$_5$ and R$_6$ are each independently a hydrogen atom, a cyano group, an alkoxycarbonyl group or a C$_1$ to C$_6$ alkyl group);

R1 and R$_2$ are a hydrogen atom, a C$_1$ to C$_8$ alkyl group, [which may be substituted with C$_3$ to C$_8$ cycloalkyl group, C$_1$ to C$_6$ alkoxy group, C$_2$ to C$_6$ alkylcarbonyl group, C$_1$ to C$_6$ alkylthio group, C$_1$ to C$_6$ alkylsulfinyl group, C$_1$ to C$_6$ alkylsulfonyl group, C$_1$ to C$_6$ alkylamino group, di(C$_1$ to C$_6$ alkyl)amino group, hydroxyl group, cyano group, C$_1$ to C$_6$ alkoxycarbonyl group, C$_1$ to C$_6$ alkylaminocarbonyl group, di(C$_1$ to C$_6$ alkyl)aminocarbonyl group, (C$_1$ to C$_6$ alkylthio)carbonyl group, carboxyl group, optionally substituted benzyloxy group, optionally substituted phenoxy group, or optionally substituted phenyl group], a C$_3$ to C$_8$ cycloalkyl group, a C$_1$ to C$_6$ alkoxycarbonyl group, a C$_3$ to C$_6$ alkylaminocarbonyl group, a di(C$_1$ to C$_6$ alkyl)aminocarbonyl group, or a (C$_1$ to C$_6$ alkylthio)carbonyl group, carboxyl group or optionally substituted) phenyl group, or, R$_1$ and R$_2$ may form a C$_2$ to C$_7$ spiro ring together with the carbon atom to which they bond;

R$_3$ and R$_4$ are a hydrogen atom, a C$_1$ to C$_6$ alkyl group (which may be substituted with 1 to 3 same or different halogen atoms, C$_3$ to C$_8$ cycloalkyl groups or C$_1$ to C$_6$ alkoxy groups) or a C$_3$ to C$_8$ cycloalkyl group, and R$_3$ and R$_4$ may form a C$_3$ to C$_7$ spiro ring together with the carbon atom to which they bond, or, R$_1$, R$_2$, R$_3$ and R$_4$ may form a 5- to 8-membered ring together with the carbon atoms to which they bond;

Y is a hydrogen atom, a C$_1$ to C$_6$ alkoxycarbonyl group, a carboxyl group, a C$_2$ to C$_6$ alkenyl group, a C$_3$ to C$_{10}$ alkyl group [which may be substituted with 1 to 3 same or different halogen atoms, C$_1$ to C$_6$ alkoxy groups, C$_2$ to C$_6$ alkenyloxy groups, C$_2$ to C$_6$ alkynyloxy groups, optionally substituted benzyloxy gorups, C$_1$ to C$_6$ alkoxycarbonyl groups, carboxyl groups, hydroxyl groups or forunyl groups], or a phenyl group substituted with 1 to 5 same or different R$_7$s;

each R$_7$ is a hydrogen atom, a C$_1$ to C$_6$ alkyl group [which may be substituted with 1 to 3 same or different halogen atoms, C$_1$ to C$_6$ alkoxy groups, hydroxyl groups, C$_1$ to C$_6$ alkylthio groups, C$_1$ to C$_6$ alkylsulfinyl groups, C$_1$ to C$_6$ alkylsulfonyl groups, C$_1$ to C$_6$ alkylamino groups, di(C$_1$ to C$_6$)alkylamino groups, cyano groups or optionally substituted phenoxy groups], a C$_1$ to C$_6$ alkoxy group (which may be substituted with 1 to 3 same or different halogen atoms, C$_1$ to C$_6$ alkoxy groups, C$_2$ to C$_6$ alkenyl groups, C$_2$ to C$_6$ alkynyl groups, C$_1$ to C$_6$ alkoxycarbonyl groups, C$_2$ to C$_7$ alkylcarbonyl groups or C$_3$ to C$_8$ cycloalkyl groups), a C$_2$ to C$_6$ alkenyl group, a C$_3$ to C$_8$ cycloalkyloxy group, a C$_1$ to C$_8$ alkylthio group (which may be substituted with 1 to 3 same or different halogen atoms or C$_1$ to C$_6$ alkoxy groups), a C$_1$ to C$_6$ alkylsulfinyl group (which may be substituted with 1 to 3 same or different halogen atoms or C$_1$ to C$_6$ alkoxy groups), a C$_1$ to C$_6$ alkylsulfonyl group (which may be substituted with 1 to 3 same or different halogen atoms or C$_1$ to C$_6$ alkoxy groups), an optionally substituted benzyloxy group, an amino group [which may be substituted with C$_1$ to C$_6$ alkyl group, C$_1$ to C$_6$ alkylsulfonyl group, C$_2$ to C$_6$ alkylcarboxyl (C$_2$ to C$_6$ alkyl) group or C$_1$ to C$_6$ alkylsulfonyl (C$_1$ to C$_6$ alkyl) group], a di(C$_1$ to C$_6$ alkyl) amino group, a halogen atom, a cyano group, a nitro group, a C$_1$ to C$_6$ alkoxycarbonyl group, a C$_3$ to C$_8$ cycloalkyloxycarbonyl group, a carboxyl group, a C$_2$ to C$_6$ alkenyloxycarbonyl group, a C$_2$ to C$_6$ alkynyloxycarbonyl group, an optionally substituted benzyloxycarbonyl group, an optionally substituted phenoxycarbonyl group or a C$_1$ to C$_6$ alkylcarbonyloxy group).

(2) a herbicide containing, as the active ingredient, an isoxazoline derivative or its salt set forth in the above (1).

BEST MODE FOR CARRYING OUT THE INVENTION

The definitions of the terms used in the present specification are given below.

"Halogen atom" refers to a fluorine atom, a chlorine atom, a bromine atom or an iodine atom.

"Alkyl group" refers to a C$_1$ to C$_{10}$ straight or branched chain alkyl group unless other wise specified; and there can be mentioned, for example, methyl group, ethyl group, n-propyl group, isopropyl group, n-butyl group, isobutyl group, sec-butyl group, tert-butyl group, n-pentyl group, isopentyl group, neopentyl group, n-hexyl group, isohexyl group, 3,3-dimethylbutyl group, heptyl group and octyl group.

"Cycloalkyl group" refers to a C_3 to C_8 cycloalkyl group; and there can be mentioned, for example, cyclopropyl group, cyclobutyl group, cyclopentyl group and cyclohexyl group.

"Alkoxy group" refers to an (alkyl)-O- group wherein the alkyl moiety has the above definition; and there can be mentioned, for example, methoxy group and ethoxy group.

"Alkylthio group", "alkylsulfinyl group" and "alkylsulfonyl group" refer, respectively, to an (alkyl)-S— group, an (alkyl)-SO— group and an (alkyl)-SO_2— group, in each of which the alkyl moiety has the above definition; and there can be mentioned, for example, methylthio group, ethylthio group, methylsulfinyl group, methylsulfonyl group and ethylsulfonyl group.

"Alkenyl group" refers to a C_2 to C_6 straight or branched chain alkenyl group; and there can be mentioned, for example, ethenyl group, 1-propenyl group, 2-propenyl group, isopropenyl group, 1-butenyl group, 2-butenyl group, 3-butenyl group and 2-pentenyl group.

"Alkynyl group" refers to a C_2 to C_6 straight or branched chain alkynyl group; and there can be mentioned, for example, ethinyl group, 2-propynyl group, 2-butinyl group and 3-butinyl group.

"Alkenyloxy group" and "alkynyloxy group" refer, respectively, to an (alkenyl)-O— group and an (alkynyl)-O— group, in each of which the alkenyl or alkynyl moiety has the above definition; and there can be mentioned, for example, 2-propenyloxy group and 2-propynyloxy group.

"Alkylamino group" and "dialkylamino group" refer, respectively, to an (alkyl)-NH— group and an $(alkyl)_2$—N— group, in each of which the alkyl moiety has the above definition; and there can be mentioned, for example, methylamino group, ethylamino group and dimethylamino group.

"Alkylcarbonyl group", "(alkylthio)carbonyl group", "alkoxycarbonyl group", "alkylaminocarbonyl group" and "dialkylaminocarbonyl group" refer, respectively, to an (alkyl)-CO— group, an (alkylthio)-CO— group, an (alkoxy)-CO— group, an (alkylamino)-CO— group and a (dialkylamino)-CO— group, in each of which the alkyl, alkylthio, alkoxy, alkylamino or dialkylamino moiety has the above definition; and there can be mentioned, for example, acetyl group, methylthiocarbonyl group, ethoxycarbonyl group, methoxycarbonyl group, methylaminocarbonyl group and dimethylaminocarbonyl group.

"Alkylaminocarbonylamino group", "dialkylaminocarbonylamino group" and "alkoxycarbonylamino group" refer, respectively, to an (alkylaminocarbonyl)-NH— group, a (dialkylaminocarbonyl)-NH— group and an (alkxrycarbonyl)-NH— group, in each of which the alkylaminocarbonyl, dialkylaminocarbonyl or alkoxycarbonyl moicty has the above definition; and there can be mentioned, for example, methylaminocarbonylamino group, dimethylaminocarbonylamino group and methoxycarbonylamino group.

"Optionally substituted phenyl group" includes phenyl groups each having, on the phenyl ring, 1 to 5 substituents such as halogen atom(s), C_1 to C_6 alkyl group(s), C_1 to C_6 alkoxy group(s) and the like.

"Optionally substituted phenoxy group" includes phenoxy groups each having, on the phenyl ring, 1 to 5 substituents such as halogen atom (s), C_1 to C_6 alkyl group(s), C_1 to C_6 alkoxy group(s) and the like.

"Optionally substituted benzyloxy group" includes benzyloxy groups each having, on the phenyl ring and at the benzylic position, 1 to 7 substituents such as halogen atom (s), C_1 to C_6 alkyl group(s), C_1 to C_6 alkoxy group(s) and the like.

"Optionally substituted phenoxycarbonyl group" includes phenoxycarbonyl groups each having, on the phenyl ring, 1 to 5 substituents such as halogen atom(s), C_1 to C_6 alkyl group(s), C_1 to C_6 alkoxy group(s) and the like.

"Salt" refers to a salt between the carboxyl group, sulfonyl group, hydroxyl group, amino group or other group present in the compound of the general formula [1] and a metal, an organic base, an organic acid or an inorganic acid. As the metal, there can be mentioned alkali metals such as sodium, potassium and the like, and alkaline earth metals such as magnesium, calcium and the like. As the organic base, there can be mentioned triethylamine, diisopropylamine, etc. As the organic acid, there can be mentioned acetic acid, oxalic acid, maleic acid, p-toluenesulfonic acid, etc. As the inorganic acid, there can be mentioned hydrochloric acid, sulfuric acid, nitric acid, etc.

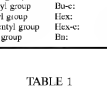
Preferable examples of the compound of the general formula [I] are those compounds wherein R_1 and R_2 are a C_1 to C_3 alkyl group or a C_1 to C_3 alkoxyalkyl group, R_3 and R_4 are a hydrogen atom or a C_1 to C_3 alkyl group, Q is a group represented by $—S(O)_n-(CR_aR_b)_m-$, R_a and R_b are a hydrogen atom or a C_1 to C_3 alkyl group, n is 2, m is 1, and Y is an optionally substituted phenyl group or a C_2 to C_{10} alkyl group.

Next, representative examples of the present compound of the general formula [1] are shown in Tables 1 to 24. However, the present compound is not restricted to these. Incidentally, the No. of each compound is used also in the later description.

The following abbreviations used in the following tables indicate the following groups.

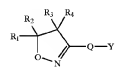
Me:	methyl group	Et:	ethyl group
Pr:	n-propyl group	Pr-i:	isopropyl group
Pr-c:	cyclopropyl group	Bu:	n-butyl group
Bu-i:	isobutyl group	Bu-s:	sec-butyl group
Bu-t:	tert-butyl group	Bu-c:	cyclobutyl group
Pen:	n-pentyl group	Hex:	n-hexyl group
Pen-c:	cyclopentyl group	Hex-c:	cyclohexyl group
Ph:	phenyl group	Bn:	benzyl group

TABLE 1



Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (°C.) or refractive index (n_D^{20})
1-1	Me	Me	H	H	SO_2CH_2	Ph	108.5–110
1-2	Me	Me	H	H	SO_2CH_2	Ph(2-Cl)	71–72
1-3	Me	Me	H	H	SO_2CH_2	Ph(3-Cl)	91.5–92
1-4	Me	Me	H	H	SO_2CH_2	Ph(4-Cl)	138–138.5
1-5	Me	Me	H	H	SO_2CH_2	Ph(2-Me)	96–97
1-6	Me	Me	H	H	SO_2CH_2	Ph(3-Me)	78–79
1-7	Me	Me	H	H	SO_2CH_2	Ph(4-Me)	97–98
1-8	Me	Me	H	H	SO_2CH_2	Ph(2-Et)	1.5390
1-9	Me	Me	H	H	SO_2CH_2	Ph(3-Et)	
1-10	Me	Me	H	H	SO_2CH_2	Ph(4-Et)	
1-11	Me	Me	H	H	SO_2CH_2	Ph(2-Pr)	
1-12	Me	Me	H	H	SO_2CH_2	Ph(3-Pr)	
1-13	Me	Me	H	H	SO_2CH_2	Ph(4-Pr)	
1-14	Me	Me	H	H	SO_2CH_2	Ph(2-Pr-i)	
1-15	Me	Me	H	H	SO_2CH_2	Ph(3-Pr-i)	
1-16	Me	Me	H	H	SO_2CH_2	Ph(4-Pr-i)	

TABLE 1-continued



Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-17	Me	Me	H	H	SO_2CH_2	Ph(2-Bu)	
1-18	Me	Me	H	H	SO_2CH_2	Ph(3-Bu)	
1-19	Me	Me	H	H	SO_2CH_2	Ph(4-Bu)	
1-20	Me	Me	H	H	SO_2CH_2	Ph(2-Bu-i)	

TABLE 2

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-21	Me	Me	H	H	SO_2CH_2	Ph(3-Bu-t)	
1-22	Me	Me	H	H	SO_2CH_2	Ph(4-Bu-t)	
1-23	Me	Me	H	H	SO_2CH_2	Ph(2-Bu-s)	
1-24	Me	Me	H	H	SO_2CH_2	Ph(3-Bu-s)	

TABLE 2-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-25	Me	Me	H	H	SO_2CH_2	Ph(4-Bu-s)	
1-26	Me	Me	H	H	SO_2CH_2	Ph(2-Bu-t)	
1-27	Me	Me	H	H	SO_2CH_2	Ph(3-Bu-t)	
1-28	Me	Me	H	H	SO_2CH_2	Ph(4-Bu-t)	
1-29	Me	Me	H	H	SO_2CH_2	Ph(2-Hex)	
1-30	Me	Me	H	H	SO_2CH_2	Ph(3-Hex)	
1-31	Me	Me	H	H	SO_2CH_2	Ph(4-Hex)	
1-32	Me	Me	H	H	SO_2CH_2	Ph(2-F)	102–103
1-33	Me	Me	H	H	SO_2CH_2	Ph(3-F)	105–105.5
1-34	Me	Me	H	H	SO_2CH_2	Ph(4-F)	138–138.5
1-35	Me	Me	H	H	SO_2CH_2	Ph(2-Br)	77–78
1-36	Me	Me	H	H	SO_2CH_2	Ph(3-Br)	
1-37	Me	Me	H	H	SO_2CH_2	Ph(4-Br)	
1-38	Me	Me	H	H	SCH_2	Ph(2,6-F_2)	77–80
1-39	Me	Me	H	H	SO_2CH_2	Ph(2,6-F_2)	110–111
1-40	Me	Me	H	H	SO_2CH_2	Ph(2-OMe)	94–95
1-41	Me	Me	H	H	SO_2CH_2	Ph(3-OMe)	89–90
1-42	Me	Me	H	H	SO_2CH_2	Ph(4-OMe)	122–124
1-43	Me	Me	H	H	SO_2CH_2	Ph(2-OEt)	76–79
1-44	Me	Me	H	H	SO_2CH_2	Ph(3-OEt)	
1-45	Me	Me	H	H	SO_2CH_2	Ph(4-OEt)	

TABLE 3

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-46	Me	Me	H	H	SO_2CH_2	Ph(2-OPr)	67–68
1-47	Me	Me	H	H	SO_2CH_2	Ph(3-OPr)	
1-48	Me	Me	H	H	SO_2CH_2	Ph(4-OPr)	
1-49	Me	Me	H	H	SO_2CH_2	Ph(2-OPr-i)	73–74
1-50	Me	Me	H	H	SO_2CH_2	Ph(3-OPr-i)	
1-51	Me	Me	H	H	SO_2CH_2	Ph(4-OPr-i)	
1-52	Me	Me	H	H	SO_2CH_2	Ph(2-OHex)	
1-53	Me	Me	H	H	SO_2CH_2	Ph(3-OHex)	
1-54	Me	Me	H	H	SO_2CH_2	Ph(4-OHex)	
1-55	Me	Me	H	H	SO_2CH_2	Ph(2-$OCHF_2$)	80–81
1-56	Me	Me	H	H	SO_2CH_2	Ph(3-$OCHF_2$)	51–53
1-57	Me	Me	H	H	SO_2CH_2	Ph(4-$OCHF_2$)	
1-58	Me	Me	H	H	SO_2CH_2	Ph(2-OCF_3)	1.492
1-59	Me	Me	H	H	SO_2CH_2	Ph(3-OCF_3)	82–84
1-60	Me	Me	H	H	SO_2CH_2	Ph(4-OCF_3)	
1-61	Me	Me	H	H	SO_2CH_2	Ph(2-OCH_2CH_2OMe)	
1-62	Me	Me	H	H	SO_2CH_2	Ph(3-OCH_2CH_2OMe)	
1-63	Me	Me	H	H	SO_2CH_2	Ph(4-OCH_2CH_2OMe)	
1-64	Me	Me	H	H	SO_2CH_2	Ph(2-SMe)	
1-65	Me	Me	H	H	SO_2CH_2	Ph(3-SMe)	
1-66	Me	Me	H	H	SO_2CH_2	Ph(4-SMe)	
1-67	Me	Me	H	H	SO_2CH_2	Ph(2-SEt)	
1-68	Me	Me	H	H	SO_2CH_2	Ph(3-SEt)	
1-69	Me	Me	H	H	SO_2CH_2	Ph(4-SEt)	
1-70	Me	Me	H	H	SO_2CH_2	Ph(2-SPr)	

TABLE 4

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-71	Me	Me	H	H	SO_2CH_2	Ph(3-SPr)	
1-72	Me	Me	H	H	SO_2CH_2	Ph(4-SPr)	
1-73	Me	Me	H	H	SO_2CH_2	Ph(2-SBu)	
1-74	Me	Me	H	H	SO_2CH_2	Ph(3-SBu)	
1-75	Me	Me	H	H	SO_2CH_2	Ph(4-SBu)	
1-76	Me	Me	H	H	SO_2CH_2	Ph(2-SHex)	
1-77	Me	Me	H	H	SO_2CH_2	Ph(3-SHex)	
1-78	Me	Me	H	H	SO_2CH_2	Ph(4-SHex)	
1-79	Me	Me	H	H	SO_2CH_2	Ph(2-$SCHF_2$)	
1-80	Me	Me	H	H	SO_2CH_2	Ph(3-$SCHF_2$)	
1-81	Me	Me	H	H	SO_2CH_2	Ph(4-$SCHF_2$)	
1-82	Me	Me	H	H	SO_2CH_2	Ph(2-SCH_2CH_2OMe)	
1-83	Me	Me	H	H	SO_2CH_2	Ph(3-SCH_2CH_2OMe)	
1-84	Me	Me	H	H	SO_2CH_2	Ph(4-SCH_2CH_2OMe)	
1-85	Me	Me	H	H	SO_2CH_2	Ph(2-SOMe)	
1-86	Me	Me	H	H	SO_2CH_2	Ph(3-SOMe)	
1-87	Me	Me	H	H	SO_2CH_2	Ph(4-SOMe)	
1-88	Me	Me	H	H	SO_2CH_2	Ph(2-SOEt)	
1-89	Me	Me	H	H	SO_2CH_2	Ph(3-SOEt)	
1-90	Me	Me	H	H	SO_2CH_2	Ph(4-SOEt)	
1-91	Me	Me	H	H	SO_2CH_2	Ph(2-SOPr)	
1-92	Me	Me	H	H	SO_2CH_2	Ph(3-SOPr)	
1-93	Me	Me	H	H	SO_2CH_2	Ph(4-SOPr)	
1-94	Me	Me	H	H	SO_2CH_2	Ph(2-SOBu)	
1-95	Me	Me	H	H	SO_2CH_2	Ph(3-SOBu)	

TABLE 5

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-96	Me	Me	H	H	SO_2CH_2	Ph(4-SOBu)	
1-97	Me	Me	H	H	SO_2CH_2	Ph(2-SOHex)	
1-98	Me	Me	H	H	SO_2CH_2	Ph(3-SOHex)	
1-99	Me	Me	H	H	SO_2CH_2	Ph(4-SOHex)	
1-100	Me	Me	H	H	SO_2CH_2	Ph(2-$SOCH_2CF_3$)	
1-101	Me	Me	H	H	SO_2CH_2	Ph(3-$SOCH_2CF_3$)	
1-102	Me	Me	H	H	SO_2CH_2	Ph(4-$SOCH_2CF_3$)	
1-103	Me	Me	H	H	SO_2CH_2	Ph(2-$SOCH_2CH_2OMe$)	
1-104	Me	Me	H	H	SO_2CH_2	Ph(3-$SOCH_2CH_2OMe$)	
1-105	Me	Me	H	H	SO_2CH_2	Ph(4-$SOCH_2CH_2OMe$)	
1-106	Me	Me	H	H	SO_2CH_2	Ph(2-SO_2Me)	97-98
1-107	Me	Me	H	H	SO_2CH_2	Ph(3-SO_2Me)	
1-108	Me	Me	H	H	SO_2CH_2	Ph(4-SO_2Me)	
1-109	Me	Me	H	H	SO_2CH_2	Ph(2-SO_2Et)	
1-110	Me	Me	H	H	SO_2CH_2	Ph(3-SO_2Et)	
1-111	Me	Me	H	H	SO_2CH_2	Ph(4-SO_2Et)	
1-112	Me	Me	H	H	SO_2CH_2	Ph(2-SO_2Pr)	
1-113	Me	Me	H	H	SO_2CH_2	Ph(3-SO_2Pr)	
1-114	Me	Me	H	H	SO_2CH_2	Ph(4-SO_2Pr)	
1-115	Me	Me	H	H	SO_2CH_2	Ph(2-SO_2Bu)	
1-116	Me	Me	H	H	SO_2CH_2	Ph(3-SO_2Bu)	
1-117	Me	Me	H	H	SO_2CH_2	Ph(4-SO_2Bu)	
1-118	Me	Me	H	H	SO_2CH_2	Ph(2-SO_2Hex)	
1-119	Me	Me	H	H	SO_2CH_2	Ph(3-SO_2Hex)	
1-120	Me	Me	H	H	SO_2CH_2	Ph(4-SO_2Hex)	

TABLE 6

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-121	Me	Me	H	H	SO_2CH_2	Ph(2-$SO_2CH_2CH_2OMe$)	
1-122	Me	Me	H	H	SO_2CH_2	Ph(3-$SO_2CH_2CH_2OMe$)	
1-123	Me	Me	H	H	SO_2CH_2	Ph(4-$SO_2CH_2CH_2OMe$)	

TABLE 6-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-124	Me	Me	H	H	SO_2CH_2	Ph(2-$SO_2CH_2CF_3$)	
1-125	Me	Me	H	H	SO_2CH_2	Ph(3-$SO_2CH_2CF_3$)	
1-126	Me	Me	H	H	SO_2CH_2	Ph(4-$SO_2CH_2CF_3$)	
1-127	Me	Me	H	H	SO_2CH_2	Ph(2-CH_2OPh)	
1-128	Me	Me	H	H	SO_2CH_2	Ph(3-CH_2OPh)	
1-129	Me	Me	H	H	SO_2CH_2	Ph(4-CH_2OPh)	
1-130	Me	Me	H	H	SO_2CH_2	Ph(2-CH_2OPh(2-Cl))	
1-131	Me	Me	H	H	SO_2CH_2	Ph(3-CH_2OPh(3-Me))	
1-132	Me	Me	H	H	SO_2CH_2	Ph(4-CH_2OPh(4-OMe))	
1-133	Me	Me	H	H	SO_2CH_2	Ph(2-NHMe)	
1-134	Me	Me	H	H	SO_2CH_2	Ph(3-NHMe)	
1-135	Me	Me	H	H	SO_2CH_2	Ph(4-NHMe)	
1-136	Me	Me	H	H	SO_2CH_2	Ph(2-$N(Me)_2$)	
1-137	Me	Me	H	H	SO_2CH_2	Ph(3-$N(Me)_2$)	
1-138	Me	Me	H	H	SO_2CH_2	Ph(4-$N(Me)_2$)	
1-139	Me	Me	H	H	SO_2CH_2	Ph(2-CN)	120–122
1-140	Me	Me	H	H	SO_2CH_2	Ph(3-CN)	
1-141	Me	Me	H	H	SO_2CH_2	Ph(4-CN)	
1-142	Me	Me	H	H	SO_2CH_2	Ph(2-NO_2)	102–103
1-143	Me	Me	H	H	SO_2CH_2	Ph(3-NO_2)	
1-144	Me	Me	H	H	SO_2CH_2	Ph(4-NO_2)	
1-145	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2Me)	97–98

TABLE 7

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-146	Me	Me	H	H	SO_2CH_2	Ph(3-CO_2Me)	
1-147	Me	Me	H	H	SO_2CH_2	Ph(4-CO_2Me)	
1-148	Me	Me	H	H	SO_2CH_2	Ph(2-$NHSO_2Me$)	
1-149	Me	Me	H	H	SO_2CH_2	Ph(3-$NHSO_2Me$)	
1-150	Me	Me	H	H	SO_2CH_2	Ph(4-$NHSO_2Me$)	
1-151	Me	Me	H	H	SO_2CH_2	Ph(2-$NHCH_2COMe$)	
1-152	Me	Me	H	H	SO_2CH_2	Ph(3-$NHCH_2COMe$)	
1-153	Me	Me	H	H	SO_2CH_2	Ph(4-$NHCH_2COMe$)	
1-154	Me	Me	H	H	SO_2CH_2	Ph(2-$NHCH_2SO_2Me$)	
1-155	Me	Me	H	H	SO_2CH_2	Ph(3-$NHCH_2SO_2Me$)	
1-156	Me	Me	H	H	SO_2CH_2	Ph(4-$NHCH_2SO_2Me$)	
1-157	Me	Me	H	H	SO_2CH_2	Ph(2-CF_3)	1.5009
1-158	Me	Me	H	H	SO_2CH_2	Ph(3-CF_3)	103–104
1-159	Me	Me	H	H	SO_2CH_2	Ph(4-CF_3)	
1-160	Me	Me	H	H	SO_2CH_2	Ph(2-CH_2OMe)	1.5352
1-161	Me	Me	H	H	SO_2CH_2	Ph(3-CH_2OMe)	
1-162	Me	Me	H	H	SO_2CH_2	Ph(4-CH_2OMe)	
1-163	Me	Me	H	H	SO_2CH_2	Ph(2-CH_2OH)	
1-164	Me	Me	H	H	SO_2CH_2	Ph(3-CH_2OH)	
1-165	Me	Me	H	H	SO_2CH_2	Ph(4-CH_2OH)	
1-166	Me	Me	H	H	SO_2CH_2	Ph(2-CH_2SMe)	
1-167	Me	Me	H	H	SO_2CH_2	Ph(3-CH_2SMe)	
1-168	Me	Me	H	H	SO_2CH_2	Ph(4-CH_2SMe)	
1-169	Me	Me	H	H	SO_2CH_2	Ph(2-CH_2SOMe)	
1-170	Me	Me	H	H	SO_2CH_2	Ph(3-CH_2SOMe)	

TABLE 8

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-171	Me	Me	H	H	SO_2CH_2	Ph(4-CH_2SOMe)	
1-172	Me	Me	H	H	SO_2CH_2	Ph(2-CH_2SO_2Me)	
1-173	Me	Me	H	H	SO_2CH_2	Ph(3-CH_2SO_2Me)	
1-174	Me	Me	H	H	SO_2CH_2	Ph(4-CH_2SO_2Me)	
1-175	Me	Me	H	H	SO_2CH_2	Ph(2-CH_2NHMe)	
1-176	Me	Me	H	H	SO_2CH_2	Ph(3-CH_2NHMe)	

TABLE 8-continued

Compound No.	R_1	R_2	H	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-177	Me	Me	H	H	SO_2CH_2	Ph(4-CH_2NHMe)	
1-178	Me	Me	H	H	SO_2CH_2	Ph(2-$CH_2N(Me)_2$)	
1-179	Me	Me	H	H	SO_2CH_2	Ph(3-$CH_2N(Me)_2$)	
1-180	Me	Me	H	H	SO_2CH_2	Ph(4-$CH_2N(Me)_2$)	
1-181	Me	Me	H	H	SO_2CH_2	Ph(2-CH_2CN)	
1-182	Me	Me	H	H	SO_2CH_2	Ph(3-CH_2CN)	
1-183	Me	Me	H	H	SO_2CH_2	Ph(4-CH_2CN)	
1-184	Me	Me	H	H	SO_2CH_2	Ph(2-F, 3-Cl)	128–130
1-185	Me	Me	H	H	SO_2CH_2	Ph(2,6-Me_2)	110–112
1-186	Me	Me	H	H	SO_2CH_2	Ph(2-OEt, 3-Me)	1.5231
1-187	Me	Me	H	H	SO_2CH_2	Ph(2-F, 3-Me)	91–92
1-188	Me	Et	H	H	SO_2CH_2	Ph	38–39
1-189	Me	Et	H	H	SO_2CH_2	Ph(2-F)	65–67
1-190	Me	Et	H	H	SO_2CH_2	Ph(3-F)	58–59
1-191	Me	Et	H	H	SO_2CH_2	Ph(4-F)	75–78
1-192	Me	Et	H	H	SO_2CH_2	Ph(2-Cl)	1.5472
1-193	Me	Et	H	H	SO_2CH_2	Ph(3-Cl)	67–68
1-194	Me	Et	H	H	SO_2CH_2	Ph(4-Cl)	93–94
1-195	Me	Et	H	H	SO_2CH_2	Ph(2-Br)	1.5289

TABLE 9

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-196	Me	Et	H	H	SO_2CH_2	Ph(3-Br)	
1-197	Me	Et	H	H	SO_2CH_2	Ph(4-Br)	
1-198	Me	Et	H	H	SCH_2	Ph(2,6-F_2)	51–52
1-199	Me	Et	H	H	$SOCH_2$	Ph(2,6-F_2)	<30
1-200	Me	Et	H	H	SO_2CH_2	Ph(2,6-F_2)	64–65
1-201	Me	Et	H	H	SO_2CH_2	Ph(2-Me)	1.5371
1-202	Me	Et	H	H	SO_2CH_2	Ph(3-Me)	41–42
1-203	Me	Et	H	H	SO_2CH_2	Ph(4-Me)	43–44
1-204	Me	Et	H	H	SO_2CH_2	Ph(2-Et)	
1-205	Me	Et	H	H	SO_2CH_2	Ph(3-Et)	
1-206	Me	Et	H	H	SO_2CH_2	Ph(4-Et)	
1-207	Me	Et	H	H	SO_2CH_2	Ph(2-Pr)	
1-208	Me	Et	H	H	SO_2CH_2	Ph(3-Pr)	
1-209	Me	Et	H	H	SO_2CH_2	Ph(4-Pr)	
1-210	Me	Et	H	H	SO_2CH_2	Ph(2-Pr-i)	
1-211	Me	Et	H	H	SO_2CH_2	Ph(3-Pr-i)	
1-212	Me	Et	H	H	SO_2CH_2	Ph(4-Pr-i)	
1-213	Me	Et	H	H	SO_2CH_2	Ph(2-Bu)	
1-214	Me	Et	H	H	SO_2CH_2	Ph(3-Bu)	
1-215	Me	Et	H	H	SO_2CH_2	Ph(4-Bu)	
1-216	Me	Et	H	H	SO_2CH_2	Ph(2-Bu-i)	
1-217	Me	Et	H	H	SO_2CH_2	Ph(3-Bu-i)	
1-218	Me	Et	H	H	SO_2CH_2	Ph(4-Bu-i)	
1-219	Me	Et	H	H	SO_2CH_2	Ph(2-Bu-s)	
1-220	Me	Et	H	H	SO_2CH_2	Ph(3-Bu-s)	

TABLE 10

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-221	Me	Et	H	H	SO_2CH_2	Ph(4-Bu-s)	
1-222	Me	Et	H	H	SO_2CH_2	Ph(2-Bu-t)	
1-223	Me	Et	H	H	SO_2CH_2	Ph(3-Bu-t)	

TABLE 10-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-224	Me	Et	H	H	SO_2CH_2	Ph(4-Bu-t)	
1-225	Me	Et	H	H	SO_2CH_2	Ph(2-Hex)	
1-226	Me	Et	H	H	SO_2CH_2	Ph(3-Hex)	
1-227	Me	Et	H	H	SO_2CH_2	Ph(4-Hex)	
1-228	Me	Et	H	H	SO_2CH_2	Ph(2-OMe)	Unable to measure
1-229	Me	Et	H	H	SO_2CH_2	Ph(3-OMe)	1.5219
1-230	Me	Et	H	H	SO_2CH_2	Ph(4-OMe)	72–74
1-231	Me	Et	H	H	SO_2CH_2	Ph(2-OEt)	
1-232	Me	Et	H	H	SO_2CH_2	Ph(3-OEt)	
1-233	Me	Et	H	H	SO_2CH_2	Ph(4-OEt)	
1-234	Me	Et	H	H	SO_2CH_2	Ph(2-OPr)	
1-235	Me	Et	H	H	SO_2CH_2	Ph(3-OPr)	
1-236	Me	Et	H	H	SO_2CH_2	Ph(4-OPr)	
1-237	Me	Et	H	H	SO_2CH_2	Ph(2-OPr-i)	
1-238	Me	Et	H	H	SO_2CH_2	Ph(3-OPr-i)	
1-239	Me	Et	H	H	SO_2CH_2	Ph(4-OPr-i)	
1-240	Me	Et	H	H	SO_2CH_2	Ph(2-OHex)	
1-241	Me	Et	H	H	SO_2CH_2	Ph(3-OHex)	
1-242	Me	Et	H	H	SO_2CH_2	Ph(4-OHex)	
1-243	Me	Et	H	H	SO_2CH_2	Ph(2-$OCHF_2$)	
1-244	Me	Et	H	H	SO_2CH_2	Ph(3-$OCHF_2$)	
1-245	Me	Et	H	H	SO_2CH_2	Ph(4-$OCHF_2$)	

TABLE 11

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-246	Me	Et	H	H	SO_2CH_2	Ph(2-OCF_3)	
1-247	Me	Et	H	H	SO_2CH_2	Ph(3-OCF_3)	
1-248	Me	Et	H	H	SO_2CH_2	Ph(4-OCF_3)	
1-249	Me	Et	H	H	SO_2CH_2	Ph(2-OCH_2CH_2OMe)	
1-250	Me	Et	H	H	SO_2CH_2	Ph(3-OCH_2CH_2OMe)	
1-251	Me	Et	H	H	SO_2CH_2	Ph(4-OCH_2CH_2OMe)	
1-252	Me	Et	H	H	SO_2CH_2	Ph(2-SMe)	
1-253	Me	Et	H	H	SO_2CH_2	Ph(3-SMe)	
1-254	Me	Et	H	H	SO_2CH_2	Ph(4-SMe)	
1-255	Me	Et	H	H	SO_2CH_2	Ph(2-SEt)	
1-256	Me	Et	H	H	SO_2CH_2	Ph(3-SEt)	
1-257	Me	Et	H	H	SO_2CH_2	Ph(4-SEt)	
1-258	Me	Et	H	H	SO_2CH_2	Ph(2-SPr)	
1-259	Me	Et	H	H	SO_2CH_2	Ph(3-SPr)	
1-260	Me	Et	H	H	SO_2CH_2	Ph(4-SPr)	
1-261	Me	Et	H	H	SO_2CH_2	Ph(2-SBu)	
1-262	Me	Et	H	H	SO_2CH_2	Ph(3-SBu)	
1-263	Me	Et	H	H	SO_2CH_2	Ph(4-SBu)	
1-264	Me	Et	H	H	SO_2CH_2	Ph(2-SHex)	
1-265	Me	Et	H	H	SO_2CH_2	Ph(3-SHex)	
1-266	Me	Et	H	H	SO_2CH_2	Ph(4-SHex)	
1-267	Me	Et	H	H	SO_2CH_2	Ph(2-$SCHF_2$)	
1-268	Me	Et	H	H	SO_2CH_2	Ph(3-$SCHF_2$)	
1-269	Me	Et	H	H	SO_2CH_2	Ph(4-$SCHF_2$)	
1-270	Me	Et	H	H	SO_2CH_2	Ph(2-SCH_2CH_2OMe)	

TABLE 12

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-271	Me	Et	H	H	SO_2CH_2	Ph(3-SCH_2CH_2OMe)	
1-272	Me	Et	H	H	SO_2CH_2	Ph(4-SCH_2CH_2OMe)	
1-273	Me	Et	H	H	SO_2CH_2	Ph(2-SOMe)	
1-274	Me	Et	H	H	SO_2CH_2	Ph(3-SOMe)	
1-275	Me	Et	H	H	SO_2CH_2	Ph(4-SOMe)	
1-276	Me	Et	H	H	SO_2CH_2	Ph(2-SOEt)	
1-277	Me	Et	H	H	SO_2CH_2	Ph(3-SOEt)	
1-278	Me	Et	H	H	SO_2CH_2	Ph(4-SOEt)	
1-279	Me	Et	H	H	SO_2CH_2	Ph(2-SOPr)	
1-280	Me	Et	H	H	SO_2CH_2	Ph(3-SOPr)	
1-281	Me	Et	H	H	SO_2CH_2	Ph(4-SOPr)	
1-282	Me	Et	H	H	SO_2CH_2	Ph(2-SOBu)	
1-283	Me	Et	H	H	SO_2CH_2	Ph(3-SOBu)	
1-284	Me	Et	H	H	SO_2CH_2	Ph(4-SOBu)	
1-285	Me	Et	H	H	SO_2CH_2	Ph(2-SOHex)	
1-286	Me	Et	H	H	SO_2CH_2	Ph(3-SOHex)	
1-287	Me	Et	H	H	SO_2CH_2	Ph(4-SOHex)	
1-288	Me	Et	H	H	SO_2CH_2	Ph(2-$SOCH_2CF_3$)	
1-289	Me	Et	H	H	SO_2CH_2	Ph(3-$SOCH_2CF_3$)	
1-290	Me	Et	H	H	SO_2CH_2	Ph(4-$SOCH_2CF_3$)	
1-291	Me	Et	H	H	SO_2CH_2	Ph(2-$SOCH_2CH_2OMe$)	
1-292	Me	Et	H	H	SO_2CH_2	Ph(3-$SOCH_2CH_2OMe$)	
1-293	Me	Et	H	H	SO_2CH_2	Ph(4-$SOCH_2CH_2OMe$)	
1-294	Me	Et	H	H	SO_2CH_2	Ph(2-SO_2Me)	
1-295	Me	Et	H	H	SO_2CH_2	Ph(3-SO_2Me)	

TABLE 13

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-296	Me	Et	H	H	SO_2CH_2	Ph(4-SO_2Me)	
1-297	Me	Et	H	H	SO_2CH_2	Ph(2-SO_2Et)	
1-298	Me	Et	H	H	SO_2CH_2	Ph(3-SO_2Et)	

TABLE 13-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-299	Me	Et	H	H	SO_2CH_2	Ph(4-SO_2Et)	
1-300	Me	Et	H	H	SO_2CH_2	Ph(2-SO_2Pr)	
1-301	Me	Et	H	H	SO_2CH_2	Ph(3-SO_2Pr)	
1-302	Me	Et	H	H	SO_2CH_2	Ph(4-SO_2Pr)	
1-303	Me	Et	H	H	SO_2CH_2	Ph(2-SO_2Bu)	
1-304	Me	Et	H	H	SO_2CH_2	Ph(3-SO_2Bu)	
1-305	Me	Et	H	H	SO_2CH_2	Ph(4-SO_2Bu)	
1-306	Me	Et	H	H	SO_2CH_2	Ph(2-SO_2Hex)	
1-307	Me	Et	H	H	SO_2CH_2	Ph(3-SO_2Hex)	
1-308	Me	Et	H	H	SO_2CH_2	Ph(4-SO_2Hex)	
1-309	Me	Et	H	H	SO_2CH_2	Ph(2-$SO_2CH_2CF_3$)	
1-310	Me	Et	H	H	SO_2CH_2	Ph(3-$SO_2CH_2CF_3$)	
1-311	Me	Et	H	H	SO_2CH_2	Ph(4-$SO_2CH_2CF_3$)	
1-312	Me	Et	H	H	SO_2CH_2	Ph(2-$SO_2CH_2CH_2OMe$)	
1-313	Me	Et	H	H	SO_2CH_2	Ph(3-$SO_2CH_2CH_2OMe$)	
1-314	Me	Et	H	H	SO_2CH_2	Ph(4-$SO_2CH_2CH_2OMe$)	
1-315	Me	Et	H	H	SO_2CH_2	Ph(2-OBn)	
1-316	Me	Et	H	H	SO_2CH_2	Ph(3-OBn)	
1-317	Me	Et	H	H	SO_2CH_2	Ph(4-OBn)	
1-318	Me	Et	H	H	SO_2CH_2	Ph(2-OBn(2-Cl))	
1-319	Me	Et	H	H	SO_2CH_2	Ph(2-OBn(3-Me))	
1-320	Me	Et	H	H	SO_2CH_2	Ph(2-OBn(4-OMe))	

TABLE 14

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-321	Me	Et	H	H	SO_2CH_2	Ph(2-NHMe)	
1-322	Me	Et	H	H	SO_2CH_2	Ph(3-NHMe)	
1-323	Me	Et	H	H	SO_2CH_2	Ph(4-NHMe)	
1-324	Me	Et	H	H	SO_2CH_2	Ph(2-$N(Me)_2$)	
1-325	Me	Et	H	H	SO_2CH_2	Ph(3-$N(Me)_2$)	
1-326	Me	Et	H	H	SO_2CH_2	Ph(4-$N(Me)_2$)	
1-327	Me	Et	H	H	SO_2CH_2	Ph(2-CN)	
1-328	Me	Et	H	H	SO_2CH_2	Ph(3-CN)	83–84
1-329	Me	Et	H	H	SO_2CH_2	Ph(4-CN)	87–89
1-330	Me	Et	H	H	SO_2CH_2	Ph(2-NO_2)	
1-331	Me	Et	H	H	SO_2CH_2	Ph(3-NO_2)	115–117
1-332	Me	Et	H	H	SO_2CH_2	Ph(4-NO_2)	

TABLE 14-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-333	Me	Et	H	H	SO_2CH_2	Ph(2-CO_2Me)	
1-334	Me	Et	H	H	SO_2CH_2	Ph(3-CO_2Me)	1.5152
1-335	Me	Et	H	H	SO_2CH_2	Ph(4-CO_2Me)	
1-336	Me	Et	H	H	SO_2CH_2	Ph(2-CF_3)	1.5021
1-337	Me	Et	H	H	SO_2CH_2	Ph(3-CF_3)	
1-338	Me	Et	H	H	SO_2CH_2	Ph(4-CF_3)	
1-339	Me	Et	H	H	SO_2CH_2	Ph(2-CH_2OMe)	
1-340	Me	Et	H	H	SO_2CH_2	Ph(3-CH_2OMe)	
1-341	Me	Et	H	H	SO_2CH_2	Ph(4-CH_2OMe)	
1-342	Me	Et	H	H	SO_2CH_2	Ph(2-CH_2OH)	
1-343	Me	Et	H	H	SO_2CH_2	Ph(3-CH_2OH)	
1-344	Me	Et	H	H	SO_2CH_2	Ph(4-CH_2OH)	
1-345	Me	Et	H	H	SO_2CH_2	Ph(2-CH_2SMe)	

TABLE 15

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-346	Me	Et	H	H	SO_2CH_2	Ph(3-CH_2SMe)	
1-347	Me	Et	H	H	SO_2CH_2	Ph(4-CH_2SMe)	
1-348	Me	Et	H	H	SO_2CH_2	Ph(2-CH_2SOMe)	
1-349	Me	Et	H	H	SO_2CH_2	Ph(3-CH_2SOMe)	
1-350	Me	Et	H	H	SO_2CH_2	Ph(4-CH_2SOMe)	
1-351	Me	Et	H	H	SO_2CH_2	Ph(2-CH_2SO_2Me)	
1-352	Me	Et	H	H	SO_2CH_2	Ph(3-CH_2SO_2Me)	
1-353	Me	Et	H	H	SO_2CH_2	Ph(4-CH_2SO_2Me)	
1-354	Me	Et	H	H	SO_2CH_2	Ph(2-CH_2NHMe)	

TABLE 15-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-355	Me	Et	H	H	SO_2CH_2	Ph(3-CH_2NHMe)	
1-356	Me	Et	H	H	SO_2CH_2	Ph(4-CH_2NHMe)	
1-357	Me	Et	H	H	SO_2CH_2	Ph(2-$CH_2N(Me)_2$)	
1-358	Me	Et	H	H	SO_2CH_2	Ph(3-$CH_2N(Me)_2$)	
1-359	Me	Et	H	H	SO_2CH_2	Ph(4-$CH_2N(Me)_2$)	
1-360	Me	Et	H	H	SO_2CH_2	Ph(2-CH_2CN)	
1-361	Me	Et	H	H	SO_2CH_2	Ph(3-CH_2CN)	
1-362	Me	Et	H	H	SO_2CH_2	Ph(4-CH_2CN)	
1-363	Et	Et	H	H	$SOCH_2$	Ph(2,6-F_2)	63–65
1-364	Et	Et	H	H	SO_2CH_2	Ph(2,6-F_2)	87–89
1-365	Me	Pr	H	H	$SOCH_2$	Ph(2,6-F_2)	44–47
1-366	Me	Pr	H	H	SO_2CH_2	Ph(2,6-F_2)	61–63
1-367	Me	Pr-i	H	H	$SOCH_2$	Ph(2,6-F_2)	1.5319
1-368	Me	Pr-i	H	H	SO_2CH_2	Ph(2,6-F_2)	62–63
1-369	Me	Me	H	H	$SO_2CH(Me)$	Ph	
1-370	Me	Me	H	H	$SO_2CH(Me)$	Ph(2,6-F_2)	

TABLE 16

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-371	Me	Et	H	H	$SO_2CH(Me)$	Ph	
1-372	Me	Et	H	H	$SO_2CH(Me)$	Ph(2,6-F_2)	
1-373	Me	Me	H	H	$SO_2C(Me)_2$	Ph	
1-374	Me	Me	H	H	$SO_2C(Me)_2$	Ph(2,6-F_2)	
1-375	Me	Et	H	H	$SO_2C(Me)_2$	Ph	
1-376	Me	Et	H	H	$SO_2C(Me)_2$	Ph(2,6-F_2)	
1-377	Me	Bu	H	H	SO_2CH_2	Ph(2,6-F_2)	111–113
1-378	Me	Pr-c	H	H	SO_2CH_2	Ph(2,6-F_2)	49–51
1-379	Me	CH_2Pr-c	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-380	—$(CH_2)_2$—		H	H	SO_2CH_2	Ph(2,6-F_2)	137–138
1-381	—$(CH_2)_3$—		H	H	SCH_2	Ph(2,6-F_2)	93–95
1-382	—$(CH_2)_3$—		H	H	SO_2CH_2	Ph(2,6-F_2)	115–115.5
1-383	—$(CH_2)_4$—		H	H	SO_2CH_2	Ph(2,6-F_2)	113–114
1-384	—$(CH_2)_5$—		H	H	SO_2CH_2	Ph(2,6-F_2)	118–120
1-385	H	—$(CH_2)_3$—		H	SO_2CH_2	Ph(2,6-F_2)	
1-386	H	—$(CH_2)_4$—		H	SCH_2	Ph(2,6-F_2)	1.5529
1-387	H	—$(CH_2)_4$—		H	SO_2CH_2	Ph(2,6-F_2)	1.5342
1-388	H	—$(CH_2)_5$—		H	SO_2CH_2	Ph(2,6-F_2)	138–139
1-389	Me	CH_2CO_2Me	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-390	Me	CH_2CO_2Et	H	H	SO_2CH_2	Ph(2,6-F_2)	1.516
1-391	Me	CH_2CN	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-392	Me	CH_2OH	H	H	SCH_2	Ph(2,6-F_2)	73–75
1-393	Me	CH_2OH	H	H	$SOCH_2$	Ph(2,6-F_2)	80–84
1-394	Me	CH_2OH	H	H	SO_2CH_2	Ph(2,6-F_2)	120–131
1-395	Me	CH_2OMe	H	H	SCH_2	Ph(2,6-F_2)	1.5279

TABLE 17

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-396	Me	CH_2OMe	H	H	$SOCH_2$	Ph(2,6-F_2)	1.5293
1-397	Me	CH_2OMe	H	H	SO_2CH_2	Ph(2,6-F_2)	105–106
1-398	Me	$CH_2OPh(2,6\text{-}Cl_2)$	H	H	SCH_2	Ph(2,6-F_2)	1.5715
1-399	Me	$CH_2OPh(2,6\text{-}Cl_2)$	H	H	$SOCH_2$	Ph(2,6-F_2)	1.5674
1-400	Me	$CH_2OPh(2,6\text{-}Cl_2)$	H	H	SO_2CH_2	Ph(2,6-F_2)	1.5461
1-401	Me	$CH_2OBz(2,6\text{-}F_2)$	H	H	SO_2CH_2	Ph(2,6-F_2)	1.5257
1-402	Me	CH_2SMe	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-403	Me	CH_2SEt	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-404	Me	CH_2SPr	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-405	Me	CH_2SPr-i	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-406	Me	CH_2SOMe	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-407	Me	CH_2SOEt	H	H	SO_2CH_2	Ph(2,6-F_2)	

TABLE 17-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-408	Me	CH_2SOPr	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-409	Me	CH_2SOPr-i	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-410	Me	CH_2NHMe	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-411	Me	CH_2NHEt	H	H	SCH_2	Ph(2,6-F_2)	1.5268
1-412	Me	CH_2NHEt	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-413	Me	CH_2NHPr	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-414	Me	CH_2NHPr-i	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-415	Me	$CH_2N(Me)_2$	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-416	Me	Bn(2-Me)	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-417	Me	Bn(3-OMe)	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-418	Me	Bn(4-Cl)	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-419	Me	CO_2H	H	H	SCH_2	Ph(2,6-F_2)	107–108
1-420	Me	CO_2Me	H	H	SCH_2	Ph(2,6-F_2)	75–76

TABLE 18

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-421	Me	CO_2Me	H	H	$SOCH_2$	Ph(2,6-F_2)	58–59
1-422	Me	CO_2Me	H	H	SO_2CH_2	Ph(2,6-F_2)	115–116
1-423	Me	CO_2Et	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-424	Me	CO_2Pr	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-425	Me	CO_2Pr-i	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-426	Me	COSMe	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-427	Me	COSEt	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-428	Me	COSPr	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-429	Me	COSPr-i	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-430	Me	CONHMe	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-431	Me	CONHEt	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-432	Me	CONHPr	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-433	Me	CONHPr-i	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-434	Me	$CON(Me)_2$	H	H	SCH_2	Ph(2,6-F_2)	1.5423
1-435	Me	$CON(Me)_2$	H	H	$SOCH_2$	Ph(2,6-F_2)	1.5409
1-436	Me	$CON(Me)_2$	H	H	SO_2CH_2	Ph(2,6-F_2)	1.5236
1-437	Me	CON(Et)(Me)	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-438	Me	$CON(Pr)_2$	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-439	Me	$CON(Pr)_2$	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-440	Me	Ph	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-441	Me	Ph(2-Me)	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-442	Me	Ph(3-OMe)	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-443	Me	Ph(4-Cl)	H	H	$SOCH_2$	Ph(2,6-F_2)	1.5788
1-444	Me	Ph(4-Cl)	H	H	SO_2CH_2	Ph(2,6-F_2)	100–101
1-445	Me	Me	Me	Me	$SOCH_2$	Ph(2,6-F_2)	

TABLE 19

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-446	Me	Me	Me	Me	SO_2CH_2	Ph(2,6-F_2)	
1-447	H	H	Me	Me	$SOCH_2$	Ph(2,6-F_2)	
1-448	H	H	Me	Me	SO_2CH_2	Ph(2,6-F_2)	
1-449	Me	Me	H	H	$SO_2CH(CO_2Me)$	Ph(2,6-F_2)	
1-450	Me	Me	H	H	$SO_2CH(CN)$	Ph(2,6-F_2)	
1-451	Me	Me	H	H	$SO_2(CH_2)_2$	Ph(2,6-F_2)	
1-452	Me	Me	H	H	$SO_2(CH_2)_3$	Ph(2,6-F_2)	
1-453	Me	Et	H	H	$SO_2CH(CO_2Me)$	Ph(2,6-F_2)	
1-454	Me	Et	H	H	$SO_2CH(CN)$	Ph(2,6-F_2)	
1-455	Me	Et	H	H	$SO_2(CH_2)_2$	Ph(2,6-F_2)	
1-456	Me	Et	H	H	$SO_2(CH_2)_3$	Ph	63–64
1-457	Me	Et	H	H	$SO_2(CH_2)_3$	Ph	1.5161
1-458	Me	Et	H	H	$SO_2(CH_2)_3$	Ph(2,6-F_2)	
1-459	Me	Me	H	H	SO_2CH_2	CF_3	
1-460	Me	Me	H	H	SO_2CH_2	CH_2CF_2	

TABLE 19-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-461	Me	Me	H	H	SO_2CH_2	CH_2OH	
1-462	Me	Me	H	H	SCH_2	CH_2OH	
1-463	Me	Me	H	H	SO_2CH_2	CH_2OMe	
1-464	Me	Me	H	H	SO_2CH_2	CH_2OHex	
1-465	Me	Me	H	H	SO_2CH_2	$CH_2OCH_2CH{=}CH_2$	
1-466	Me	Me	H	H	SO_2CH_2	CH_2OBn	
1-467	Me	Et	H	H	SCH_2	CO_2H	1.5088
1-468	Me	Et	H	H	SO_2CH_2	CH_2CO_2Me	1.4852
1-469	Me	Et	H	H	SCH_2	CH_2CO_2Me	1.4919
1-470	Me	Me	H	H	SO_2CH_2	CH_2CO_2Me	

TABLE 20

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-471	Me	Me	H	H	SO_2CH_2	CH_2CO_2H	
1-472	Me	Me	H	H	SO_2CH_2	CH_2OH	
1-473	Me	Me	H	H	SO_2CH_2	CH_2CHO	
1-474	Me	Me	H	H	SO_2CH_2	$CH{=}CH_2$	
1-475	Me	Et	H	H	SO_2CH_2	CF_3	
1-476	Me	Et	H	H	SO_2CH_2	CH_2CF_3	
1-477	Me	Et	H	H	SCH_2	CH_2OH	1.5088
1-478	Me	Et	H	H	SO_2CH_2	CH_2OH	
1-479	Me	Et	H	H	SO_2CH_2	CH_2OMe	
1-480	Me	Et	H	H	SO_2CH_2	CH_2OHex	
1-481	Me	Et	H	H	SO_2CH_2	$CH_2OCH_2CH{=}CH_2$	
1-482	Me	Et	H	H	SO_2CH_2	CH_2OBn	
1-483	Me	Et	H	H	SO_2CH_2	CH_2CO_2Me	
1-484	Me	Et	H	H	SO_2CH_2	CH_2CO_2Hex	
1-485	Me	Et	H	H	SO_2CH_2	CH_2OH	
1-486	Me	Et	H	H	SO_2CH_2	CH_2CHO	
1-487	Me	Me	H	H	SO_2CH_2	Ph(2,3-Cl_2)	128–129
1-488	Me	Me	H	H	SO_2CH_2	Ph(2,4-Cl_2)	122–123
1-489	Me	Me	H	H	SO_2CH_2	Ph(2,5-Cl_2)	123–124
1-490	Me	Me	H	H	SO_2CH_2	Ph(2,6-Cl_2)	153–154
1-491	Me	Me	H	H	SO_2CH_2	Ph(3,4-Cl_2)	121–122
1-492	Me	Me	H	H	SO_2CH_2	Ph(3,5-Cl_2)	103–104
1-493	Me	—$(CH_2)_4$—		H	SO_2CH_2	Ph(2,6-F_2)	95–97
1-494	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-F)	108–109
1-495	Me	Me	Me	H	SO_2CH_2	Ph(2,6-F_2)	1.5183

TABLE 21

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-496	Me	H	Me	H	SO_2CH_2	Ph(2,6-F_2)	64–65
1-497	Me	Me	H	H	SO_2CH_2	Ph(3,4-F_2)	109–110
1-498	Me	Me	H	H	SO_2CH_2	Ph(2,5-F_2)	107–108
1-499	Me	Me	H	H	SO_2CH_2	Ph(2-F,6-NO_2)	146–147
1-500	Me	Me	H	H	SO_2CH_2	Ph(2,4,6-F3)	87–88
1-501	Me	Me	H	H	SO_2CH_2	Ph(2,3,6-F3)	136–138
1-502	Me	Me	H	H	SO_2CH_2	Ph(2,6-Et_2)	51–53
1-503	Me	Me	H	H	SO_2CH_2	Ph(2-NO_2, 3-CO-Me)	112–114
1-504	Me	Me	H	H	SO_2CH_2	Ph(2,3-F_2)	124–125
1-505	Me	Me	H	H	SO_2CH_2	Ph(2,4-F_2)	104–105
1-506	Me	Me	H	H	SO_2CH_2	Ph(3,5-F_2)	136–140
1-507	Me	Me	H	H	SO_2CH_2	Ph(2,3,4-F_3)	100–103
1-508	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-F_3)	105–107
1-509	Me	Me	H	H	SO_2CH_2	Ph(3,4,5-F_3)	150–151
1-510	Me	Me	H	H	SO_2CH_2	Ph(2,4,5-F_3)	121–126
1-511	Me	Me	H	H	SO_2CH_2	Ph(2,4-Me_2)	1.5421
1-512	Me	Me	H	H	SO_2CH_2	Ph(2,5-Me_2)	65–66
1-513	Me	Me	H	H	SO_2CH_2	Ph(3,4-Me_2)	62–65

TABLE 21-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-514	Me	Me	H	H	SO_2CH_2	Ph(2-F, 5-CF_3)	95–97
1-515	Me	Me	H	H	SO_2CH_2	Ph(2-F, 3-CF_3)	109–111
1-516	Me	Me	H	H	SO_2CH_2	Ph(2-F, 4-Br)	123–125
1-517	Me	Me	H	H	SO_2CH_2	Ph(2-SO_2CF_3)	80–81
1-518	H	—$(CH_2)_3$—		H	SO_2CH_2	Ph(2,6-F_2)	65–66
1-519	H	—$(CH_2)_4$—		H	SO_2CH_2	Ph(2,6-F_2)	97–99
1-520	Pr-c	Pr-c	H	H	SO_2CH_2	Ph(2,6-F_2)	95–96

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TABLE 22

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-521	Me	Me	H	H	SO_2CH_2	Ph(2-I)	70–72
1-522	Me	Me	H	H	SO_2CH_2	Ph(2,3-Me_2)	123–124
1-523	Me	Me	H	H	SO_2CH_2	Ph(3,5-Me_2)	97–98
1-524	Me	Me	H	H	SO_2CH_2	Ph(3,5-OMe_2)	125–126
1-525	Me	Me	H	H	SO_2CH_2	Ph(2-Et, 6-Me)	1.5414
1-526	Me	Me	H	H	SO_2CH_2	Ph(2-OEt, 6-F)	1.5251
1-527	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CF_3)	69–90
1-528	Me	Me	H	H	SO_2CH_2	Ph(2-F, 4-CF_3)	124–125
1-529	Me	Me	H	H	SO_2CH_2	Ph(2,4,6-Me_3)	119–120
1-530	Me	Me	H	H	SO_2CH_2	Ph(2-OMe, 5-NO_2)	125–126
1-531	Me	Me	H	H	SO_2CH_2	Ph(2,3,4,5,6-F_5)	113–114

TABLE 22-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-532	Me	H	H	H	SO_2CH_2	Ph(2,6-F_2)	126–127
1-533	H	H	H	H	SO_2CH_2	Ph(2,6-F_2)	125–126
1-534	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-OMe)	125–127
1-535	Me	Me	H	H	SO_2CH_2	Ph(2,6-OMe_2)	165–167
1-536	Me	Me	H	H	SO_2CH_2	Ph(2,6-OEt_2)	85–88
1-537	Me	Me	H	H	SO_2CH_2	Ph(2-Me, 3-NO_2)	109–111
1-538	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 4-F)	92–93
1-539	Me	Me	H	H	SO_2CH_2	Ph(4-Cl, 2-NO_2)	136–137
1-540	Me	Me	H	H	SO_2CH_2	Ph(5-Me, 2-NO_2)	124–125
1-541	Me	Me	H	H	SO_2CH_2	Ph(4-F, 3-CF_3)	99–101
1-542	Me	Me	H	H	SO_2CH_2	Ph(3-F, 5-CF_3)	87–89
1-543	Me	Me	H	H	SO_2CH_2	Ph(3,5-$(CF_3)_2$)	130–132
1-544	Me	Me	H	H	SO_2CH_2	Ph(2,5-$(CF_2)_2$)	100–103
1-545	Me	Me	H	H	SO_2CH_2	Ph(3,5-Br_2)	115–116

TABLE 23

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-546	Me	Me	H	H	SO_2CH_2	Ph(3,5-$(NO_2)_2$)	162–163
1-547	Me	Me	H	H	SO_2CH_2	Ph(2,3,5,6-$(Me)_4$)	128–130
1-548	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-I)	137–138
1-549	Me	Me	H	H	SO_2CH_2	Ph(2-NH_2, 6-F)	118–121
1-550	Me	Me	H	H	SO_2CH_2	Ph(2,6-F_2, 3-Me)	118–119
1-551	Me	Me	H	H	SO_2CH_2	Ph(2-F, 3-CF_3)	50–51
1-552	Me	Me	H	H	SO_2CH_2	Ph(2-NH_2)	107–109
1-553	Me	Me	H	H	SO_2CH_2	Ph(2-Pr, 6-F)	126–127
1-554	Me	Me	H	H	SO_2CH_2	Ph(2,6-Br_2)	158–160
1-555	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CO_2Me)	103–105
1-556	Me	—$(CH_2)_5$—		H	SO_2CH_2	Ph(2,6-F_2)	86–87
1-557	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-NMe_2)	108–110
1-558	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-NEt_2)	90–92
1-559	Me	Me	H	H	SO_2CH_2	Ph(2-OCH$_2$C≡CH)	110–113
1-560	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-Me)	98–100
1-561	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-$OCHF_2$)	83–84
1-562	Me	Pr-c	H	H	SO_2CH_2	Ph(2-$OCHF_2$)	1.5215
1-563	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-OMe)	128–129
1-564	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-OEt)	65–67
1-565	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-OPr-n)	66–68
1-566	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-OPr-i)	1.5402
1-567	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-OCH_2CF_3)	92–95
1-568	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-OBu-n)	50–51

TABLE 23-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-569	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-OPr-n)	74–76.5
1-570	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-OPr-i)	1.5139

TABLE 24

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-571	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-OBu-n)	74–75
1-572	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-OBu-i)	92–94
1-573	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-$OCHF_2$)	1.4961
1-574	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-OBu-n)	65–67
1-575	Me	Me	H	H	SO_2CH_2	Ph(2-CF_3)	1.4965
1-576	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-OCH_2C═CH)	102–105
1-577	Me	Me	H	H	SO_2CH_2	Ph(2-OCH_2CO_2-Me)	110–111
1-578	Me	Me	H	H	SO_2CH_2	Ph(2-OCH_2CO_2-Et)	92–93
1-579	Me	Me	H	H	SO_2CH_2	Ph(2-O$(CH_2)_2$OMe)	1.5089
1-580	Me	Me	H	H	SO_2CH_2	Ph(2-O$(CH_2)_2$OPh)	1.4961
1-581	Me	Me	H	H	$SO_2CH(Me)$	Ph	120–121
1-582	Me	Me	H	H	$SCH(Me)$	Ph	59–60
1-583	Me	Me	H	H	SO_2CH_2	Ph(2-Me, 6-MeO)	92–93
1-584	Me	Me	H	H	SO_2CH_2	Ph(2-Me, 3-Pr-i, 6-MeO)	108–109
1-585	Me	Me	H	H	SO_2CH_2	Ph(2-OEt,6-CF_3)	88–89
1-586	Me	Me	H	H	SO_2CH_2	Ph(2-CH_2OEt)	1.5318
1-587	Me	Me	H	H	SO_2CH_2	Ph(2-OCOMe)	87–89
1-588	Me	Me	H	H	SO_2CH_2	Ph(2-OCH_2Ph)	120–123
1-589	Me	Me	H	H	SO_2CH_2	Ph(2-OCH_2CH═CH_2)	71–73
1-590	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-OCH_2CH═CH_2)	Unable to measure
1-591	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-OCH_2C═CH)	108–111
1-592	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2H)	182–184
1-593	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2Et)	1.5332
1-594	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2Pr-n)	1.5294
1-595	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2Pr-i)	1.5252

TABLE 25

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-596	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2Bu-n)	1.5262
1-597	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2Bu-s)	1.5223
1-598	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2Bu-i)	64–65
1-599	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2CH_2CH═CH_2)	Unable to measure
1-600	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2CH_2C≡CH)	98–101
1-601	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2Pen-c)	78–79
1-602	Me	Me	H	H	SO_2CH_2	Ph(2-OEt, 6-Me)	Unable to measure
1-603	Me	Me	H	H	SO_2CH_2	Ph(2-OPr-n, 6-Me)	Unable to measure
1-604	Me	Me	H	H	SO_2CH_2	Ph(2-OPr-i, 6-Me)	1.5364
1-605	Me	Me	H	H	SO_2CH_2	Ph(2-OBu-s, 6-Me)	Unable to measure
1-606	Me	Me	H	H	SO_2CH_2	Ph(2-Me, 6-OCH_2CH═CH_2)	Unable to measure
1-607	Me	Me	H	H	SO_2CH_2	Ph(2-Me, 6-OCH_2C═CH)	Unable to measure
1-608	Me	Me	H	H	SO_2CH_2	Ph(2-OCH_2Pr-c)	1.5379
1-609	Me	Me	H	H	SO_2CH_2	Ph(2-OPen-c)	1.5409
1-610	Me	Me	H	H	SO_2CH_2	Ph(2-OHex-c)	1.5396
1-611	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2CH_2Ph)	96–97
1-612	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2CH_2Ph(2-Cl))	1.5631
1-613	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2CH_2Ph(4-Cl))	1.5661
1-614	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2CH_2Ph(4-Cl))	1.5642

TABLE 25-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-615	Me	Me	H	H	SO_2CH_2	Ph(2-CH_2OBu-n)	42–43
1-616	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-Me_3)	97–99
1-617	Me	Et	H	H	SO_2CH_2	Ph(2,3,5-Me_3)	68–70
1-618	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-CO_2Me)	136–137
1-619	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-CO_2Et)	108–109
1-620	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-CO_2Pr-n)	76–77

TABLE 26

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-621	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-CO_2Pr-i)	114–115
1-622	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-CO_2Bu-s)	94–95
1-623	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-CO_2Bu-n)	94–97
1-624	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-CO_2Bu-i)	99–100
1-625	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-CO_2CH_2Ph)	121–122
1-626	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-CO_2CH_2Ph(2-Cl))	111–112
1-627	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-CO_2CH_2Ph(3-Cl))	82–83
1-628	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-CO_2CH_2Ph(4-Cl))	111–112
1-629	H	$CON(Et)_2$	H	H	SCH_2	Ph(2,6-F_2)	1.5372
1-630	H	$CON(Et)_2$	H	H	$SOCH_2$	Ph(2,6-F_2)	1.5374
1-631	H	$CON(Et)_2$	H	H	SO_2CH_2	Ph(2,6-F_2)	1.5122
1-632	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 5-OMe)	92–93
1-633	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 5-OEt)	114–115
1-634	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 5-OPr-n)	98–99
1-635	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 5-OPr-i)	94–95
1-636	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 5-OBu-n)	87–88
1-637	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 5-OCH_2CH═CH_2)	66–67
1-638	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 5-OCH_2C≡CH)	91–92
1-639	Me	Me	H	H	SO_2CH_2	Ph(2-Et, 6-OMe)	78–79
1-640	Me	Me	H	H	SO_2CH_2	Ph(2-Cl, 6-CO_2H)	176–176.5
1-641	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CO_2H)	176–177
1-642	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CO_2Et)	67–68
1-643	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CO_2Pr-n)	55–56
1-644	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CO_2Pr-i)	92–93
1-645	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CO_2Bu-n)	94–95

TABLE 27

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-646	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CO_2Bu-s)	49–50
1-647	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CO_2Bu-i)	86–87
1-648	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CO_2CH_2Ph)	191–192
1-649	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CO_2CH_2Ph(2-Cl))	89–90
1-650	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CO_2CH_2Ph(3-Cl))	89–90
1-651	Me	Me	H	H	SO_2CH_2	Ph(2-F, 6-CO_2CH_2Ph(4-Cl))	108–109
1-652	Me	Et	H	H	SO_2CH_2	Ph(2,3,5,6-Me_4)	94–95
1-653	Me	Me	H	H	SO_2CH_2	Ph(2-OEt, 6-Et)	88–90
1-654	Me	Me	H	H	SO_2CH_2	Ph(2-OPr-n, 6-Et)	1.5321
1-655	Me	Me	H	H	SO_2CH_2	Ph(2-OPr-i, 6-Et)	1.5312
1-656	Me	Me	H	H	SO_2CH_2	Ph(2-OBu-n, 6-Et)	43–45
1-657	Me	Me	H	H	SO_2CH_2	Ph(2-OCH-CH—CH_3, 6-Et)	1.545
1-658	Me	Me	H	H	SO_2CH_2	Ph(2-OCH_2C═CH, 6-Et)	1.5489
1-659	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-Me_2F)	125–131
1-660	Me	Et	H	H	SO_2CH_2	Ph(2,3,5,6-F_4)	110–112
1-661	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2Me, 3-Me)	
1-662	Me	Et	H	H	SO_2CH_2	Ph(2-CO_2Me, 3-Me)	59–61
1-663	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2Et, 3-Me)	
1-664	Me	Et	H	H	SO_2CH_2	Ph(2-CO_2Et, 3-Me)	1.5292
1-665	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2Bu-i, 3-Me)	
1-666	Me	Et	H	H	SO_2CH_2	Ph(2-CO_2Bu-i, 3-Me)	1.5192
1-667	Me	Me	H	H	SO_2CH_2	Ph(2,5-Me_2, 6-OMe)	117–118

TABLE 27-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-568	Me	Me	H	H	SO_2CH_2	Ph(2,5-Me_2, 6-OEt)	1.5309
1-569	Me	Me	H	H	SO_2CH_2	Ph(2,5-Me_2, 6-OPr-n)	75–76
1-570	Me	Et	H	H	SO_2CH_2	Ph(2,3,5,6-$(Me)_4$)	

TABLE 28

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-571	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$, 6-OMe)	
1-572	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$, 6-OEt)	
1-573	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$, 6-OPr-n)	
1-574	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$, 6-OPr-i)	
1-575	Me	Me	H	H	SO_2CH_2	Ph(2,5-$(Me)_2$, 3,6-Cl_2)	
1-576	Me	Me	H	H	SO_2CH_2	Ph(2,5-$(Me)_2$, 3,6-Br_2)	
1-577	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$, 5-Cl)	
1-578	Me	Me	H	H	SO_2CH_2	Ph(2,3,6-$(Me)_3$, 5-Br)	
1-579	Me	Me	H	H	SCH_2	Ph	1.5521
1-580	Me	CH_2CO_2H	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-581	Me	CH_2COEt	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-582	Me	CH_2COMe	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-583	Me	$CH_2CON(Et)_2$	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-584	Me	$CH_2CON(Me)_2$	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-585	Me	$CH_2CONHEt$	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-586	Me	$CH_2CONHMe$	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-587	Me	CH_2COSEt	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-588	Me	CH_2COSMe	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-589	Me	CH_2OPh	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-590	Me	CH_2OPh(2-Me)	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-591	Me	CH_2OPh(2-OMe)	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-592	Me	CH_2OPh(3-Me)	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-593	Me	CH_2OPh(3-OMe)	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-594	Me	CH_2OPh(4-Me)	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-595	Me	CH_2OPh(4-OMe)	H	H	SO_2CH_2	Ph(2,6-F_2)	

TABLE 29

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-696	Me	CH_2SO_2Et	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-697	Me	CH_2SO_2Me	H	H	SO_2CH_2	Ph(2,6-F_2)	
1-698	Me	Et	H	H	SCH_2	Ph(2,3,4,5,6-$(Me)_5$)	
1-699	Me	Et	H	H	SCH_2	Ph(2,3,5-$(Me)_3$)	
1-700	Me	Et	H	H	SCH_2	Ph(2,3,5-$(Me)_3$, 6-OCH_2CF_3)	
1-701	Me	Et	H	H	SCH_2	Ph(2,3,5-$(Me)_3$, 6-$OCHF_2$)	
1-702	Me	Et	H	H	SCH_2	Ph(2,3,5-$(Me)_3$, 6-OEt)	
1-703	Me	Et	H	H	SCH_2	Ph(213,5-$(Me)_3$, 6-OMe)	
1-704	Me	Et	H	H	SCH_2	Ph(2,3,5-$(Me)_3$, 6-OPr-i)	
1-705	Me	Et	H	H	SCH_2	Ph(2,3,5-$(Me)_3$, 6-OPr-n)	
1-706	Me	Et	H	H	SCH_2	Ph(213,6-$(Me)_3$, 5-Br)	
1-707	Me	Et	H	H	SCH_2	Ph(2,3,6-$(Me)_3$, 5-Cl)	
1-708	Me	Et	H	H	SCH_2	Ph(2,3,6-$(Me)_3$, 5-F)	
1-709	Me	Et	H	H	SCH_2	Ph(2,3,6-$(Me)_3$, 5-I)	
1-710	Me	Et	H	H	SCH_2	Ph(2,5-$(Me)_2$, 3,6-Br_2)	
1-711	Me	Et	H	H	SCH_2	Ph(2,5-$(Me)_2$, 3,6-Cl_2)	
1-712	Me	Et	H	H	SO_2CH_2	Ph(2,3,4,5,6-$(Me)_5$)	
1-713	Me	Et	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$)	
1-714	Me	Et	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$, 6-OCH_2CF_3)	
1-715	Me	Et	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$, 6-$OCHF_2$)	
1-716	Me	Et	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$, 6-OEt)	
1-717	Me	Et	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$, 6-OMe)	
1-718	Me	Et	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$, 6-OPr-i)	
1-719	Me	Et	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$, 6-OPr-n)	
1-720	Me	Et	H	H	SO_2CH_2	Ph(2,3,6-$(Me)_3$, 5-Br)	

TABLE 30

Compound No.	R_3	R_5	R_1	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-721	Me	Et	H	H	SO_2CH_2	Ph(2,3,6-$(Me)_3$, 5-Cl)	
1-722	Me	Et	H	H	SO_2CH_2	Ph(2,3,5-$(Me)_3$, 5-F)	
1-723	Me	Et	H	H	SO_2CH_2	Ph (2,3,6-$(Me)_3$, 5-I)	
1-724	Me	Et	H	H	SO_2CH_2	Ph(2,5-$(Me)_2$, 3,6-Br_2)	
1-725	Me	Et	H	H	SO_2CH_2	Ph (2,5-$(Me)_2$, 3,6-Cl_2)	
1-726	Me	Et	H	H	$SOCH_2$	Ph(2,3,4,5,6-$(Me)_5$)	
1-727	Me	Et	H	H	$SOCH_2$	Ph(2,3,5-$(Me)_3$)	
1-728	Me	Et	H	H	$SOCH_2$	Ph(2,3,5-$(Me)_2$, 6-OCH_2CF_3)	
1-729	Me	Et	H	H	$SOCH_2$	Ph(2,3,5-$(Me)_2$, 6-$OCHF_2$)	
1-730	Me	Et	H	H	$SOCH_2$	Ph(2,3,5-$(Me)_2$, 6-OEt)	
1-731	Me	Et	H	H	$SOCH_2$	Ph(2,3,5-$(Me)_2$, 6-OMe)	
1-732	Me	Et	H	H	$SOCH_2$	Ph(2,3,5-$(Me)_2$, 6-OPr-i)	
1-733	Me	Et	H	H	$SOCH_2$	Ph(2,3,5-$(Me)_2$, 6-OPr-n)	
1-734	Me	Et	H	H	$SOCH_2$	Ph(2,3,6-$(Me)_3$, 5-Br)	
1-735	Me	Et	H	H	$SOCH_2$	Ph(2,3,6-$(Me)_3$, 5-Cl)	
1-736	Me	Et	H	H	$SOCH_2$	Ph(2,3,6-$(Me)_3$, 5-F)	
1-737	Me	Et	H	H	$SOCH_2$	Ph(2,3,6-$(Me)_3$, 5-I)	
1-738	Me	Et	H	H	$SOCH_2$	Ph(2,5-$(Me)_2$, 3,6-Br_2)	
1-739	Me	Et	H	H	$SOCH_2$	Ph(2,5-$(Me)_2$, 3,6-Cl_2)	
1-740	Me	Me	H	H	SCH_2	Ph(2,3,4,5,6-$(Me)_5$)	
1-741	Me	Me	H	H	SCH_2	Ph(2,3,5-$(Me)_3$)	
1-742	Me	Me	H	H	SCH_2	Ph(2,3,5-$(Me)_2$, 6-OCH_2CF_3)	
1-743	Me	Me	H	H	SCH_2	Ph(2,3,5-$(Me)_2$, 6-$OCHF_2$)	
1-744	Me	Me	H	H	SCH_2	Ph(2,3,5-$(Me)_2$, 6-OEt)	
1-745	Me	Me	H	H	SCH_2	Ph(2,3,5-$(Me)_2$, 6-OMe)	

TABLE 31

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-746	Me	Me	H	H	SCH_2	Ph(2,3,5-$(Me)_3$, 6-OPr-i)	
1-747	Me	Me	H	H	SCH_2	Ph(2,3,5-$(Me)_3$, 6-OPr-n)	
1-748	Me	Me	H	H	SCH_2	Ph(2,3,5,6-$(Me)_4$)	
1-749	Me	Me	H	H	SCH_2	Ph(2,3,6-$(Me)_3$, 5-Br)	
1-750	Me	Me	H	H	SCH_2	Ph(2,3,6-$(Me)_3$, 5-Cl)	
1-751	Me	Me	H	H	SCH_2	Ph(2,3,6-$(Me)_3$, 5-F)	
1-752	Me	Me	H	H	SCH_2	Ph(2,3,6-$(Me)_3$, 5-I)	
1-753	Me	Me	H	H	SCH_2	Ph(2,3-Me_2)	
1-754	Me	Me	H	H	SCH_2	Ph(2,4-Me_2)	
1-755	Me	Me	H	H	SCH_2	Ph(2,5-$(Me)_2$, 3,6-Br_2)	
1-756	Me	Me	H	H	SCH_2	Ph(2,5-$(Me)_2$, 3,6-Cl_2)	
1-757	Me	Me	H	H	SCH_2	Ph(2,5-Me_2)	
1-758	Me	Me	H	H	SCH_2	Ph(2,6-Me_2)	
1-759	Me	Me	H	H	SCH_2	Ph(2-Br)	
1-760	Me	Me	H	H	SCH_2	Ph(2-Bu)	
1-761	Me	Me	H	H	SCH_2	Ph(2-Bu-i)	
1-762	Me	Me	H	H	SCH_2	Ph(2-Bu-s)	
1-763	Me	Me	H	H	SCH_2	Ph(2-Bu-t)	
1-764	Me	Me	H	H	SCH_2	Ph(2-CF_3)	
1-765	Me	Me	H	H	SCH_2	Ph(2-Cl)	
1-766	Me	Me	H	H	SCH_2	Ph(2-Et)	
1-767	Me	Me	H	H	SCH_2	Ph(2-F)	
1-768	Me	Me	H	H	SCH_2	Ph(2-Hex)	
1-769	Me	Me	H	H	SCH_2	Ph(2-Me)	
1-770	Me	Me	H	H	SCH_2	Ph(2-OCF_3)	

TABLE 32

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-771	Me	Me	H	H	SCH_2	Ph(2-$OCHF_2$)	
1-772	Me	Me	H	H	SCH_2	Ph(2-OEt)	
1-773	Me	Me	H	H	SCH_2	Ph(2-OHex)	
1-774	Me	Me	H	H	SCH_2	Ph(2-OMe)	
1-775	Me	Me	H	H	SCH_2	Ph(2-OPr)	
1-776	Me	Me	H	H	SCH_2	Ph(2-OPr-i)	
1-777	Me	Me	H	H	SCH_2	Ph(2-Pr)	
1-778	Me	Me	H	H	SCH_2	Ph(2-Pr-i)	
1-779	Me	Me	H	H	SCH_2	Ph(3,4-Me_2)	
1-780	Me	Me	H	H	SCH_2	Ph(3,5-Me_2)	
1-781	Me	Me	H	H	SCH_2	Ph(3-Br)	
1-782	Me	Me	H	H	SCH_2	Ph(3-Bu)	
1-783	Me	Me	H	H	SCH_2	Ph(3-Bu-i)	
1-784	Me	Me	H	H	SCH_2	Ph(3-Bu-s)	
1-785	Me	Me	H	H	SCH_2	Ph(3-Bu-t)	
1-786	Me	Me	H	H	SCH_2	Ph(3-CF_3)	
1-787	Me	Me	H	H	SCH_2	Ph(3-Cl)	
1-788	Me	Me	H	H	SCH_2	Ph(3-Et)	
1-789	Me	Me	H	H	SCH_2	Ph(3-F)	
1-790	Me	Me	H	H	SCH_2	Ph(3-Hex)	
1-791	Me	Me	H	H	SCH_2	Ph(3-Me)	
1-792	Me	Me	H	H	SCH_2	Ph(3-OCF_3)	
1-793	Me	Me	H	H	SCH_2	Ph(3-$OCHF_2$)	
1-794	Me	Me	H	H	SCH_2	Ph(3-OEt)	
1-795	Me	Me	H	H	SCH_2	Ph(3-OHex)	

TABLE 33

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-796	Me	Me	H	H	SCH_2	Ph(3-OMe)	
1-797	Me	Me	H	H	SCH_2	Ph(3-OPr)	

TABLE 33-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-798	Me	Me	H	H	SCH_2	Ph(3-OPr-i)	
1-799	Me	Me	H	H	SCH_2	Ph(3-Pr)	
1-800	Me	Me	H	H	SCH_2	Ph(3-Pr-i)	
1-801	Me	Me	H	H	SCH_2	Ph(4-Br)	
1-802	Me	Me	H	H	SCH_2	Ph(4-Bu)	
1-803	Me	Me	H	H	SCH_2	Ph(4-Bu-i)	
1-804	Me	Me	H	H	SCH_2	Ph(4-Bu-s)	
1-805	Me	Me	H	H	SCH_2	Ph(4-Bu-t)	
1-806	Me	Me	H	H	SCH_2	Ph(4-CF_3)	
1-807	Me	Me	H	H	SCH_2	Ph(4-Cl)	
1-808	Me	Me	H	H	SCH_2	Ph(4-Et)	
1-809	Me	Me	H	H	SCH_2	Ph(4-F)	
1-810	Me	Me	H	H	SCH_2	Ph(4-Hex)	
1-811	Me	Me	H	H	SCH_2	Ph(4-Me)	
1-812	Me	Me	H	H	SCH_2	Ph(4-OCF_3)	
1-813	Me	Me	H	H	SCH_2	Ph(4-$OCHF_2$)	
1-814	Me	Me	H	H	SCH_2	Ph(4-OH)	
1-815	Me	Me	H	H	SCH_2	Ph(4-OHex)	
1-816	Me	Me	H	H	SCH_2	Ph(4-OMe)	
1-817	Me	Me	H	H	SCH_2	Ph(4-OPr)	
1-818	Me	Me	H	H	SCH_2	Ph(4-OPr-i)	
1-819	Me	Me	H	H	SCH_2	Ph(4-Pr)	
1-820	Me	Me	H	H	SCH_2	Ph(4-Pr-i)	

TABLE 34

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-821	Me	Me	H	H	SO_2CH_2	Ph(2,3,4,5,6-Me_5)	
1-822	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-Me_3)	
1-823	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-Me_3), 6-OCH_2CF_3)	
1-824	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-Me_3), 6-$OCHF_2$)	
1-825	Me	Me	H	H	SO_2CH_2	Ph(2,3,6-Me_3), 5-F)	
1-826	Me	Me	H	H	SO_2CH_2	Ph(2,3,6-Me_3), 5-I)	
1-827	Me	Me	H	H	$SOCH_2$	Ph(2,3,4,5,6-Me_5)	
1-828	Me	Me	H	H	$SOCH_2$	Ph(2,3,5-Me_3)	
1-829	Me	Me	H	H	$SOCH_2$	Ph(2,3,5-Me_3), 6-OCH_2CF_3)	
1-830	Me	Me	H	H	$SOCH_2$	Ph(2,3,5-Me_3), 6-$OCHF_2$)	
1-831	Me	Me	H	H	$SOCH_2$	Ph(2,3,5-Me_3), 6-OEt)	
1-832	Me	Me	H	H	$SOCH_2$	Ph(2,3,5-Me_3), 6-OMe)	
1-833	Me	Me	H	H	$SOCH_2$	Ph(2,3,5-Me_3), 6-OPr-i)	
1-834	Me	Me	H	H	$SOCH_2$	Ph(2,3,5-Me_3), 6-OPr-n)	
1-835	Me	Me	H	H	$SOCH_2$	Ph(2,3,5,6-Me_4)	
1-836	Me	Me	H	H	$SOCH_2$	Ph(2,3,6-Me_3), 5-Br)	
1-837	Me	Me	H	H	$SOCH_2$	Ph(2,3,6-Me_3), 5-Cl)	
1-838	Me	Me	H	H	$SOCH_2$	Ph(2,3,6-Me_3), 5-F)	
1-839	Me	Me	H	H	$SOCH_2$	Ph(2,3,6-Me_3), 5-I)	
1-840	Me	Me	H	H	$SOCH_2$	Ph(2,3-Me_2))	
1-841	Me	Me	H	H	$SOCH_2$	Ph(2,4-Me_2))	
1-842	Me	Me	H	H	$SOCH_2$	Ph(2,5-$(Me)_2$, 3,6-Br_2)	

TABLE 34-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-843	Me	Me	H	H	$SOCH_2$	Ph(2,5-$(Me)_2$, 3,6-Cl_2)	
1-844	Me	Me	H	H	$SOCH_2$	Ph(2,5-Me_2)	
1-845	Me	Me	H	H	$SOCH_2$	Ph(2,6-Me_2)	

TABLE 35

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-846	Me	Me	H	H	$SOCH_2$	Ph(2-Br)	
1-847	Me	Me	H	H	$SOCH_2$	Ph(2-Bu)	
1-848	Me	Me	H	H	$SOCH_2$	Ph(2-Bu-i)	
1-849	Me	Me	H	H	$SOCH_2$	Ph(2-Bu-s)	
1-850	Me	Me	H	H	$SOCH_2$	Ph(2-Bu-t)	
1-851	Me	Me	H	H	$SOCH_2$	Ph(2-CF_3)	
1-852	Me	Me	H	H	$SOCH_2$	Ph(2-Cl)	
1-853	Me	Me	H	H	$SOCH_2$	Ph(2-Et)	
1-854	Me	Me	H	H	$SOCH_2$	Ph(2-F)	
1-855	Me	Me	H	H	$SOCH_2$	Ph(2-Hex)	
1-856	Me	Me	H	H	$SOCH_2$	Ph(2-Me)	
1-857	Me	Me	H	H	$SOCH_2$	Ph(2-OCF_3)	
1-858	Me	Me	H	H	$SOCH_2$	Ph(2-$OCHF_2$)	
1-859	Me	Me	H	H	$SOCH_2$	Ph(2-OEt)	
1-860	Me	Me	H	H	$SOCH_2$	Ph(2-OHex)	
1-861	Me	Me	H	H	$SOCH_2$	Ph(2-OMe)	
1-862	Me	Me	H	H	$SOCH_2$	Ph(2-OPr)	
1-863	Me	Me	H	H	$SOCH_2$	Ph(2-OPr-i)	
1-864	Me	Me	H	H	$SOCH_2$	Ph(2-Pr)	
1-865	Me	Me	H	H	$SOCH_2$	Ph(2-Pr-i)	
1-866	Me	Me	H	H	$SOCH_2$	Ph(3,4-Me_2)	
1-867	Me	Me	H	H	$SOCH_2$	Ph(3,5-Me_2)	
1-868	Me	Me	H	H	$SOCH_2$	Ph(3-Br)	
1-869	Me	Me	H	H	$SOCH_2$	Ph(3-Bu)	
1-870	Me	Me	H	H	$SOCH_2$	Ph(3-Bu-i)	

TABLE 36

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-871	Me	Me	H	H	$SOCH_2$	Ph(3-Bu-s)	
1-872	Me	Me	H	H	$SOCH_2$	Ph(3-Bu-t)	
1-873	Me	Me	H	H	$SOCH_2$	Ph(3-CF_3)	
1-874	Me	Me	H	H	$SOCH_2$	Ph(3-Cl)	
1-875	Me	Me	H	H	$SOCH_2$	Ph(3-Et)	
1-876	Me	Me	H	H	$SOCH_2$	Ph(3-F)	
1-877	Me	Me	H	H	$SOCH_2$	Ph(3-Hex)	
1-878	Me	Me	H	H	$SOCH_2$	Ph(3-Me)	
1-879	Me	Me	H	H	$SOCH_2$	Ph(3-OCF_3)	
1-880	Me	Me	H	H	$SOCH_2$	Ph(3-$OCHF_2$)	
1-881	Me	Me	H	H	$SOCH_2$	Ph(3-OEt)	
1-882	Me	Me	H	H	$SOCH_2$	Ph(3-OHex)	
1-883	Me	Me	H	H	$SOCH_2$	Ph(3-OMe)	
1-884	Me	Me	H	H	$SOCH_2$	Ph(3-OPr)	
1-885	Me	Me	H	H	$SOCH_2$	Ph(3-OPr-i)	
1-886	Me	Me	H	H	$SOCH_2$	Ph(3-Pr)	
1-887	Me	Me	H	H	$SOCH_2$	Ph(3-Pr-i)	
1-888	Me	Me	H	H	$SOCH_2$	Ph(4-Br)	
1-889	Me	Me	H	H	$SOCH_2$	Ph(4-Bu)	
1-890	Me	Me	H	H	$SOCH_2$	Ph(4-Bu-i)	
1-891	Me	Me	H	H	$SOCH_2$	Ph(4-Bu-s)	
1-892	Me	Me	H	H	$SOCH_2$	Ph(4-Bu-t)	

TABLE 36-continued

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-893	Me	Me	H	H	$SOCH_2$	Ph(4-CF_3)	
1-894	Me	Me	H	H	$SOCH_2$	Ph(4-Cl)	
1-895	Me	Me	H	H	$SOCH_2$	Ph(4-Et)	

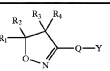
TABLE 37

Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-896	Me	Me	H	H	$SOCH_2$	Ph(4-F)	
1-897	Me	Me	H	H	$SOCH_2$	Ph(4-Hex)	
1-898	Me	Me	H	H	$SOCH_2$	Ph(4-Me)	
1-899	Me	Me	H	H	$SOCH_2$	Ph(4-OCF_3)	
1-900	Me	Me	H	H	$SOCH_2$	Ph(4-$OCHF_2$)	
1-901	Me	Me	H	H	$SOCH_2$	Ph(4-OEt)	
1-902	Me	Me	H	H	$SOCH_2$	Ph(4-OHex)	
1-903	Me	Me	H	H	$SOCH_2$	Ph(4-OMe)	
1-904	Me	Me	H	H	$SOCH_2$	Ph(4-OPr)	
1-905	Me	Me	H	H	$SOCH_2$	Ph(4-OPr-i)	
1-906	Me	Me	H	H	$SOCH_2$	Ph(4-Pr)	
1-907	Me	Me	H	H	$SOCH_2$	Ph(4-Pr-i)	
1-908	Me	Me	H	CH_2Pr-c	SO_2CH_2	Ph(2,6-F_2)	
1-909	Me	Me	H	CH_2CF_3	SO_2CH_2	Ph(2,6-F_2)	
1-910	Me	Me	H	CH_2OMe	SO_2CH_2	Ph(2,6-F_2)	
1-911	Me	Me	H	CH_2Pr-c	$SOCH_2$	Ph(2,6-F_2)	
1-912	Me	Me	H	CH_2CF_3	$SOCH_2$	Ph(2,6-F_2)	
1-913	Me	Me	H	CH_2OMe	$SOCH_2$	Ph(2,6-F_2)	
1-914	Me	Me	H	CH_2Pr-c	SCH_2	Ph(2,6-F_2)	
1-915	Me	Me	H	CH_2CF_3	SCH_2	Ph(2,6-F_2)	
1-916	Me	Me	H	CH_2OMe	SCH_2	Ph(2,6-F_2)	
1-917	Me	Me	H	Pr-c	SCH_2	Ph(2,6-F_2)	
1-918	Me	Me	H	Pr-c	$SOCH_2$	Ph(2,6-F_2)	
1-919	Me	Me	H	Pr-c	SO_2CH_2	Ph(2,6-F_2)	
1-920	Me	Me	H	H	SO_2CH_2	$CH_2OCH_2C{\equiv}CH$	

TABLE 38

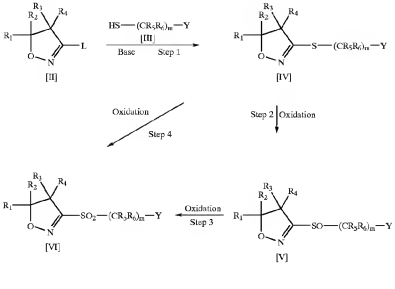
Compound No.	R_1	R_2	R_3	R_4	Q	Y	Melting point (° C.) or refractive index (n_D^{20})
1-921	Me	Me	H	H	SO_2CH_2	Ph(2-$OC_2H_4CO_2Me$)	
1-922	Me	Me	H	H	SO_2CH_2	Ph(2-OC_2H_4COMe)	
1-923	Me	Me	H	H	SO_2CH_2	Ph(2-CO_2Ph)	
1-924	Me	Me	H	H	SCH_2	Ph(2,3,5-(Me)$_3$, 6-F)	
1-925	Me	Me	H	H	SCH_2	Ph(2,3,5-(Me)$_3$, 6-Cl)	
1-926	Me	Me	H	H	SCH_2	Ph(2,3,5-(Me)$_3$, 6-Br)	
1-927	Me	Et	H	H	SCH_2	Ph(2,3,5-(Me)$_3$, 6-F)	
1-928	Me	Et	H	H	SCH_2	Ph(2,3,5-(Me)$_3$, 6-Cl)	
1-929	Me	Et	H	H	SCH_2	Ph(2,3,5-(Me)$_3$, 6-Br)	
1-930	Me	Me	H	H	$SOCH_2$	Ph(2,3,5-(Me)$_3$, 6-F)	
1-931	Me	Me	H	H	$SOCH_2$	Ph(2,3,5-(Me)$_3$, 6-Cl)	
1-932	Me	Me	H	H	$SOCH_2$	Ph(2,3,5-(Me)$_3$, 6-Br)	
1-933	Me	Et	H	H	$SOCH_2$	Ph(2,3,5-(Me)$_3$, 6-F)	
1-934	Me	Et	H	H	$SOCH_2$	Ph(2,3,5-(Me)$_3$, 6-Cl)	
1-935	Me	Et	H	H	$SOCH_2$	Ph(2,3,5-(Me)$_3$, 6-Br)	
1-936	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-(Me)$_3$, 6-F)	
1-937	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-(Me)$_3$, 6-Cl)	
1-938	Me	Me	H	H	SO_2CH_2	Ph(2,3,5-(Me)$_3$, 6-Br)	
1-939	Me	Et	H	H	SO_2CH_2	Ph(2,3,5-(Me)$_3$, 6-F)	
1-940	Me	Et	H	H	SO_2CH_2	Ph(2,3,5-(Me)$_3$, 6-Cl)	
1-941	Me	Et	H	H	SO_2CH_2	Ph(2,3,5-(Me)$_3$, 6-Br)	

TABLE 39



Compound No.	R_1	R_2	R_3	R_4	Q—Y	Melting point (° C.) or refractive index (n_D^{20})
2-1	Me	Me	H	H	SO_2Me	82–84
2-2	Me	Me	H	H	SO_2Et	59–60
2-3	Me	Me	H	H	SO_2Pr	
2-4	Me	Me	H	H	SO_2Pr-i	
2-5	Me	Me	H	H	SO_2Bu	
2-6	Me	Me	H	H	SO_2Bu-i	
2-7	Me	Me	H	H	SO_2Bu-s	
2-8	Me	Me	H	H	SO_2Bu-t	
2-9	Me	Me	H	H	SO_2Hex	
2-10	Me	Me	H	H	$SO_2C_{10}H_{21}$	
2-11	Me	Me	H	H	$SO_2C_{16}H_{33}$	
2-12	Me	Et	H	H	SO_2Me	1.4771
2-13	Me	Et	H	H	SO_2Et	1.4759
2-14	Me	Et	H	H	SO_2Pr	1.4742
2-15	Me	Et	H	H	SO_2Pr-i	1.4752
2-16	Me	Et	H	H	SO_2Bu	1.4711
2-17	Me	Et	H	H	SO_2Bu-i	1.4696
2-18	Me	Et	H	H	SO_2Bu-s	1.4750
2-19	Me	Et	H	H	SO_2Bu-t	30–31.5
2-20	Me	Et	H	H	SO_2Hex	
2-21	Me	Et	H	H	$SO_2C_{10}H_{21}$	1.4685
2-22	Me	Et	H	H	$SO_2C_{16}H_{33}$	1.4705
2-23	Me	Pr-c	H	H	SO_2CH_2	1.4921
2-24	Me	H	Me	H	SO_2Me	1.4778
2-25	Me	—$(CH_2)_4$—	H		SO_2Me	1.5116
2-26	H	—$(CH_2)_5$—	H		SO_2Me	1.5122
2-27	H	—$(CH_2)_6$—	H		SO_2Me	1.5135
2-28	—$(CH_2)_4$—		H	H	SO_2Me	65–67
2-29	—$(CH_2)_5$		H	H	SO_2Me	72–73

The present compound represeated by the general formula [I] can bc produced according to the processes shown below. However, the production process is not restricted to these. <Production Process 1>Steps 1 to 4



[wherein L is a leaving group such as halogen atom, phenylsulfonyl group which may be substituted with C_1 to C_4 alkyl group (e.g. p-toluenesulfonyl group), C_1 to C_4 alkylsulfonyl group (e.g. methylsulfonyl group) or the like (chlorine atom is preferred); and R_1, R_2, R_3, R_4, R_5, R_6, Y and m have the same definitions as given above].

The above production process is explained in detail on each step.

(Step 1)

A compound represented by the general formula [II] is reacted with a mercaptan derivative represented by the general formula [III] in the presence of a base in an appropriate solvent or without using any solvent (preferably in an appropriate solvent), or with a salt (which is a sodium salt or a potassium salt) of a mercaptan derivative represented by the general formula [III] in an appropriate solvent, whereby an intended sulfide derivative represented by the general formula [IV] can be obtained.

The solvent can be exemplified by ethers such as diethyl ether, diethoxyethane, dioxane, tetrahydrofuran (THF) and the like; halogenated hydrocarbons such as dichloromethane, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene, dichlorobenzene and the like; amides such as N,N-dimethylacetamide, N,N-dimethylformamide (DMF), N-methyl-2-pyrrolidinone and the like; sulfur compounds such as dimethyl sulfoxide (DMSO), sulfolane and the like; aromatic hydrocarbons such as benzene, toluene, xylene and the like; alcohols such as methanol, ethanol, propanol, isopropanol, butanol, tert-butanol and the like; ketones such as acetone, 2-butanone and the like; nitriles such as acetonitrile and the like; water; and mixtures thereof.

The base can be exemplified by metal hydrides such as sodium hydride and the like; alkali metal amides such as sodium amide, lithium diisopropylamide and the like; organic bases such as pyridine, triethylamine, 1,8-diazabicyclo[5.4.0]-7-undecene and the like; inorganic bases such as alkali metal hydroxide (e.g. sodium hydroxide or potassium hydroxide), alkaline earth metal hydroxide (e.g. calcium hydroxide or magnesium hydroxide), alkali metal carbonate (e.g. sodium carbonate or potassium carbonate), alkali metal bicarbonate (e.g. sodium hydrogen-

carbonate or potassium hydrogencarbonate) and the like; and alcohol metal salts such as sodium methoxide, potassium tert-butoxide and the like.

The reaction temperature is any temperature between 0° C. and the reflux temperature of the reaction system, preferably a temperature between 10 and 100° C. The reaction time differs depending upon the compounds used but is 0.5 to 24 hours.

(Step 2)

In the oxidation reaction of the sulfide derivative represented by the general formula [IV], the sulfide derivative of the general formula [IV] is reacted with an oxidizing agent (for example, an organic peroxide such as m-chloroperbenzoic acid, performic acid or peracetic acid, or an inorganic peroxide such as hydrogen peroxide, potassium permanganate or sodium periodate) in an appropriate solvent, whereby an intended sulfoxide derivative represented by the general formula [V] can be obtained.

The solvent can be exemplified by halogenated hydrocarbons such as dichloromethane, chloroform, dichloroethane, carbon tetrachloride, chlorobenzene, dichlorobenzene and the like; ethers such as dioxane, tetrahydrofuran (THF), dimethoxyethane, diethyl ether and the like; amides such as N,N-dimethylacetamide, N,N-dimethylformamide (DMF), N-methyl-2-pyrrolidinone and the like; alcohols such as methanol, ethanol, propanol, isopropanol, butanol, tert-butanol and the like; ketones such as acetone, 2-butanone and the like; nitrites such as acetonitrile and the like; acetic acid; water; and mixtures thereof.

The reaction temperature is any temperature between 0° C. and the reflux temperature of the reaction system, preferably a temperature between 10 and 60° C. The reaction time differs depending upon the compounds used but is 1 to 72 hours.

(Step 3)

The sulfoxide derivative represented by the general formula [V] is reacted with an oxidizing agent (the same as described in the step 2) in an appropriate solvent (the same as described in the step 2), whereby an intended sulfone derivative represented by the general formula [VI] can be obtained.

The reaction temperature is any temperature between 0° C. and the reflux temperature of the reaction system, preferably a temperature between 10 and 60° C. The reaction time differs depending upon the compounds used but is 1 to 72 hours.

(Step 4)

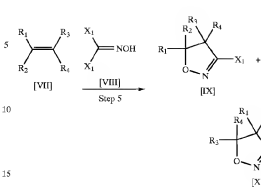
When, in the oxidation reaction of the sulfide derivative represented by the general formula [IV], the oxidizing agent is used by an appropriate amount, the sulfone derivative represented by the general formula [VI] can be obtained without isolating the sulfoxide derivative represented by the general formula [V].

That is, the sulfide derivative represented by the general formula [IV] is reacted with an oxidizing agent (the same as described in the step 2) in an appropriate solvent (the same as described in the step 2), whereby an intended sulfoxide derivative represented by the general formula [V] can be obtained.

The reaction temperature is any temperature between 0° C. and the reflux temperature of the reaction system, preferably a temperature between 10 and 60° C. The reaction time differs depending upon the compounds used but is 1 to 72 hours.

A compound represented by the genera, formula [II] wherein L is a halogen atom, can be synthesized by the following step 5.

(Step 5)



[wherein X_1 is a halogen atom (a chlorine atom is preferred), and R_1, R_2, R_3 and R_4 have the same definitions as given above].

That is, a compound represented by the general formula [VIII] is reacted with an olefin derivative represented by the general formula [VII] in the presence of a base in an appropriate solvent or without using any solvent (preferably in an appropriate solvent), whereby isoxazoline compounds represented by the general formulas [IX] and [X] can be obtained. When both R_3 and R_4 are a hydrogen atom, an isoxazoline compound represented by the general formula [IX] is obtained preferentially.

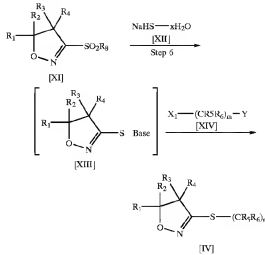
The solvent can be exemplified by ethers such as ethylene glycol dimethyl ether, ethylene glycol diethyl ether, diethyl ether, dioxane, tetrahydrofuran and the like; halogenated hydrocarbons such as dichloroethane, carbon tetrachloride, chlorobenzene, dichlorobenzene and the like; aromatic hydrocarbons such as benzene, toluene, xylene and the like; acetic acid esters such as ethyl acetate, butyl acetate and the like; water; and mixtures thereof.

The base can be exemplified by alkali metal hydroxides such as sodium hydroxide, potassium hydroxide and the like; alkaline earth metal hydroxides such as calcium hydroxide, magnesium hydroxide and the like; alkali metal carbonates such as sodium carbonate, potassium carbonate and the like; alkali metal bicarbonates such as sodium hydrogencarbonate, potassium hydrogencarbonate and the like; alkali metal acetates such as sodium acetate, potassium acetate and the like; alkali metal fluorides such as sodium fluoride, potassium fluoride and the like, and organic bases such as pyridine, triethylamine, 1,8-diazabicyclo[5.4.0]-7-undecene and the like.

The reaction temperature is any temperature between 0° C. and the reflux temperature of the reaction system, preferably a temperature between 10 and 80° C. The reaction time differs depending upon the compounds used but is 0.5 hour to 2 weeks.

Incidentally, the compound represented by the general formula [VII] used in the above step 5 as an intermidiate can be a commercial product or synthesized by a known reaction such as Wittig reaction or the like. The compound represented by the general formula [VIII] can be synthesized by, for example, a method described in Liebigs Annalen der Chemie, p. 985 (1989).

<Production Process 2>Step 6



(wherein X_1, R_1, R_2, R_3, R_4, R_5, R_6, Y and m have the same definitions as given above; R_6 is a C_1 to C_4 alkyl group or a benzyl group, preferably a lower alkyl group such as methyl group, ethyl group or the like; and base is the same as described in the step 1).

The sulfide derivative represented by the general formula [IV], described in the Production Process 1 can be obtained also by the following process.

That is, a compound represented by the general formula [XI] is reacted with sodium hydrogensulfide hydrate represented by the general formula [XII] in the presence of a base (the same as described in the step 1) in an appropriate solvent or without using any solvent (preferably in an appropriate solvent) (Reagalit may be added in some cases), whereby a mercaptan salt represented by the general formula [XIII] can be obtained in the reaction system. The reaction mixture is reacted with a halogen derivative represented by the general formula [XIV] without isolating the mercaptan salt represented by the general formula [XIII], whereby a sulfide derivative represented by the general formula [IV] can be obtained.

The solvent can be exemplified by ethers such as dioxane, tetrahydrofuran (THF) and the like; halogenated hydrocarbons such as dichloromethane, carbon tetrachloride, chlorobenzene, dichlorobenzene and the like; amides such as N,N-dimethylacetamide, N,N-dimethylformamide (DMF), N-methyl-2-pyrrolidinone and the like; sulfur compounds such as dimethyl sulfoxide (DMSO), sulfolane and the like; aromatic hydrocarbons such as benzene, toluene, xylene and the like; alcohols such as methanol, ethanol, propanol, isopropanol, butanol, tert-butanol and the like; ketones such as acetone, 2-butanone and the like; nitrites such as acetonitrile and the like; water; and mixtures thereof.

The reaction temperature is any temperature between 0° C. and the reflux temperature of the reaction system, preferably a temperature between 10 and 100° C. The reaction time differs depending upon the compounds used but is 0.5 to 24 hours.

The sulfone derivative represented by the general formula [XI] can be produced by the method shown in the Step 1 of the Production Process 1. In this case, the group $—(CR_4R_5)_m—Y$ in the general formula [III] is an alkyl group or a benzyl group.

Next, the method for producing the present compound, the method for formulation with the present compound, and the application are specifically described by way of Examples. The method for producing the intermediate for the present compound is also described.

Example 1

Production Of 3-benzylthio-5,5-dimethyl-2-isoxazoline (Present Compound No. 1-679)

To a solution of 2.8 g (22.5 mmoles) of benzylmercaptan dissolved in 50 ml of dimethylformamide were added, in a nitrogen current, 3.2 g (23.2 mmoles) of anhydrous potassium carbonate and 3.0 g (22.5 mmoles) of 3-chloro-5,5-dimethyl-2-isoxazoline. The mixture was stirred at 100° C. for 2 hours to give rise to a reaction. After the completion of the reaction, the reaction mixture was poured into water, followed by extraction with ethyl acetate. The resulting organic layer was washed with water and an aqueous sodium chloride solution in this order and then dried over anhydrous magnesium sulfate. The resulting solution was subjected to vacuum distillation to remove the solvent contained therein, and the residue was purified by silica gel column chromatography to obtain 3.1 g (yield: 62.0%) of 3-benzylthio-5,5-dimethyl-2-isoxazoline as a yellow oily substance (refractive index n_D^{20}=1.5521).

^{1}H-NMR [$CDCl_3$/TMS(ppm)]: 7.24–7.39 (5H,m), 4.26 (2H,s), 2.77 (2H,s), 1.40 (6H,s)

<Example 2>

Production Of 5-ethyl-3-(2,6-difluorobenzylsulfinyl)-5-methyl-2-isoxazoline (Present Compound No. 1-199)

To a solution of 4.1 g (15.0 mmoles) of 5-ethyl-3-(2,6-difluorobenzylthio)-5-methyl-2-isoxazoline dissolved in 50 ml of chloroform was added, with ice-cooling, 4.6 g (18.8 mmoles) of m-chloroperbenzoic acid (70%). The mixture was stirred for 1 hour and then at room temperature for 12 hours to give rise to a reaction. After the completion of the reaction, the reaction mixture was poured into water, followed by extraction with chloroform. The resulting organic phase was washed with an aqueous sodium hydrogensulfite solution, an aqueous potassium carbonate solution, water and an aqueous sodium chloride solution in this order, and then dried over anhydrous magnesium sulfate. The resulting solution was subjected to vacuum distillation to remove the solvent contained therein. The residue was purified by silica gel column chromatography (solvent system: hexane-ethyl acetate) to obtain 1.5 g (yield: 34.8%) of 5-ethyl-3-(2,6-difluorobenzylsulfinyl)-5-methyl-2-isoxazoline as a white powder (melting point: 30° C. or less).

^{1}H-NMR [$CDCl_3$/TMS(ppm)]: 7.39–7.28 (1H, m), 7.03–6.94 (2H, m), 4.38 (2H, s), 3.04 (1H, ABq, J=17.2, Δυ=85.7 Hz)+3.12 (1H, s) 1.75 (2H, m), 1.44 (3H, s)+1.41 (3H, 3), 0.97 (3H, m)

<Example 3>

Production Of 5-ethyl-3-(2,6-difluorobenzylsulfonyl)-5-methyl-2-isoxazoline (Present Compound No. 1-200)

To a solution of 0.8 g (2.8 mmoles) of 5-ethyl-3-(2,6-difluorobenzylsulfinyl)-5-methyl-2-isoxazoline dissolved in 50 ml of chloroform was added, with ice-cooling, 1.0 g (4.1 mmoles) of m-chloroperbenzoic acid. The mixture was stirred for 1 hour and then at room temperature for 12 hours to give rise to a reaction. After the completion of the

reaction, the reaction mixture was poured into water, followed by extraction with chloroform. The resulting organic phase was washed with an aqueous sodium hydrogensulfite solution, an aqueous potassium carbonate solution, water and an aqueous sodium chloride solution in this order, and then dried over anhydrous magnesium sulfate. The resulting solution was subjected to vacuum distillation to remove the solvent contained therein. The residue was purified by silica gel column chromatography (solvent system: hexane-ethyl acetate) to obtain 0.6 g (yield: 75.0%) of 5-ethyl-3-(2,6-difluorobenzylsulfonyl)-5-methyl-2-isoxazoline as a white powder (melting point: 64 to 65° C.).

^{1}H-NMR [$CDCl_3$/TMSδ(ppm)] 7.36–7.46 (1H,m), 6.98–7.04 (2H,m), 4.73 (2H,s), 3.04 (2H, ABq, J=17.2, $\Delta\nu$=51.1 Hz), 1.77 (2H,q), 1.46 (3H,s), 0.97 (3H,t)

<Example 4>

Production Of 3-(2,6-difluorobenzylsulfonyl)-5,5-dimethyl-2-isoxazoline
(Present Compound No. 1-39)

To a solution of 3.9 g (15.2 mmoles) of 3-(2,6-difluorobenzylthio)-5,5-dimethyl-2-isoxazoline dissolved in 50 ml of chloroform was added, with ice-cooling, 8.5 g (34.5 mmoles) of m-chloroperbenzoic acid. The mixture was stirred for 1 hour and then at room temperature for 12 hours to give rise to a reaction. After the completion of the reaction, the reaction mixture was poured into water, followed by extraction with chloroform. The resulting organic phase was washed with an aqueous sodium hydrogensulfite solution, an aqueous potassium carbonate solution, water and an aqueous sodium chloride solution in this order, and then dried over anhydrous magnesium sulfate. The resulting solution was subjected to vacuum distillation to remove the solvent contained therein. The residue was washed with diisopropyl ether to obtain 3.4 g (yield: 77.3%) of 3-(2,6-difluorobenzylsulfonyl)-5,5-dimethyl-2-isoxazbline as a white powder (melting point: 110 to 111° C.).

^{1}H-NMR [$CDCl_3$/TMSδ(ppm)]: 7.35–7.45 (1H,m), 6.98–7.03 (2H,m), 4.72 (2H,s), 3.06 (2H,s), 1.51 (6H,s)

<Example 5>

Production Of 3-(2,6-difluorobenzylthio)-5,5-dimethyl-2-isoxazoline
(Present Compound No. 1-38)

To a solution of 5.0 g (28.2 mmoles) of 3-methylsulfonyl-5,5-dimethyl-2-isoxazoline (present compound No. 2-1) dissolved in 50 ml of DMF were added, with ice-cooling, 4.5 g (purity: 70%, 56.1 mmoles) of sodium hydrogensulfide hydrate, 7.8 g (56.4 mmoles) of potassium carbonate and 8.7 g (56.5 mmoles) of Rongalit. The mixture was stirred for 2 hours. Thereto was added 5.8 g (28.0 mmoles) of 2,6-difluorobenzylbenzyl bromide. The mixture was stirred at room temperature for 12 hours to give rise to a reaction. After the completion of the reaction, the reaction mixture was poured into water, followed by extraction with ethyl acetate. The resulting organic phase was washed with an aqueous sodium chloride solution and then dried over anhydrous magnesium sulfate. The resulting solution was subjected to vacuum distillation to remove the solvent contained therein. The residue was purified by silica gel column chromatography (solvent system=hexane-ethyl acetate) to obtain 5.8 g (yield: 80.0%) of 3-(2,6-difluorobenzylthio)-5,5-dimethyl-2-isoxazoline as a white powder (melting point: 77 to 80° C.).

^{1}H-NMR [$CDCl_3$/TMSδ(ppm)]: 7.20–7.28 (1H,m), 6.86–6.93 (2H,m), 4.35 (2H,s), 2.81 (2H,s), 1.43 (6H,s)<

<Example 6>

Production Of 3-methylsulfonyl-5,5-dimethyl-2-isoxazoline (Present Compound No. 2-1)

To a solution of 193.0 g (1.07 M) of 3-chloro-5,5-dimethyl-2-isoxazoline dissolved in 500 ml of DMF was dropwise added, with ice-cooling, 1.0 kg (content=15%, 2.14 M) of an aqueous sodium methanethiolate solution. The mixture was stirred at room temperature for 12 hours to give rise to a reaction. After the completion of the reaction, the reaction mixture was poured into water, followed by extraction with ethyl acetate. The resulting organic phase was washed with an aqueous sodium chloride solution and then dried over anhydrous magnesium sulfate. The resulting solution was subjected to vacuum distillation to remove the solvent contained therein, to obtain 115.0 g (yield: 74.1%) of 3-methylthio-5,5-dimethyl-2-isoxazoline. This residue (141.2 mmoles) was dissolved in 1 liter of chloroform. Thereto was added, with ice-cooling, 392.0 g (1.59 M) of m-chloroperbenzoic acid (purity: 70%), followed by stirring at that temperature for 1 hour and at room temperature for 12 hours to give rise to a reaction. After the completion of the reaction, the precipitated m-chloroperbenzoic acid was removed by filtration. The filtrate was washed with an aqueous sodium hydrogensulfite solution, water, an aqueous sodium hydrogencarbonate solution and an aqueous sodium chloride solution in this order, and then dried over anhydrous magnesium sulfate. The resulting solution was subjected to vacuum distillation to remove the solvent contained therein. The residue was washed with diisopropyl ether to obtain 77.6 g (yield: 59.1%) of 3-methylsulfonyl-5,5-dimethyl-2-isoxazoline as a white powder (melting point: 82 to 84° C.).

^{1}H-NMR [$CDCl_3$/TMSδ(ppm)]: 3.26 (3H,s), 3.12 (2H,s), 1.51 (6H,s)

(Examples Of Production Of Intermediates)

<Reference Example 1>

Production Of 3-chloro-5,5-dimethyl-2-isoxazoline
(Compound IX)

534.0 g (4.0 moles) of N-chlorosuccinimide was slowly added, at 65 to 70° C., to a solution of 182.7 g (2.05 moles) of glyoxylic aldoxime dissolved in 2 liters of dimethoxyethane, followed by refluxing for 1 hour with heating. Thereto were added, with ice-cooling, 1,440.0 g (14.4 moles) of potassium hydrogencarbonate and 10 ml of water. To the mixture was added 360.0 g (6.4 moles) of 2-methylpropene, followed by stirring at room temperature for 24 hours to give rise to a reaction. The reaction mixture was poured into water, followed by extraction with isopropyl ether. The resulting organic phase was washed with water and an aqueous sodium chloride solution in this order and then dried over anhydrous magnesium sulfate. The resulting solution was subjected to vacuum distillation to remove the solvent contained therein, to obtain 107.7 g (yield: 40.0%) of 3-chloro-5,5-dimethyl-2-isoxazoline as a yellow viscous liquid.

^{1}H-NMR [$CDCl_3$/TMSδ(ppm)]: 2.93 (2H,s), 1.47 (6H,s)

<Reference Example 2>

Production Of 3-chloro-5-ethyl-5-methyl-2-isoxazoline (Compound IX)

61.9 g (463.4 mmoles) of N-chlorosuccinimide was slowly added, at 60° C., to a solution of 20.6 g (231.7

mmoles) of glyoxylic acid aldoxime dissolved in 500 ml of dimethoxyethane. Then, the mixture was refluxed for 10 minutes with heating. Thereto were added, with ice-cooling, 50 ml (463.4 mmoles) of 2-methyl-1-butene, 98.9 g (1,622 mmoles) of potassium hydrogencarbonate and 10 ml of water, followed by stirring for 12 hours to give rise to a reaction. The reaction mixture was poured into water, followed by extraction with n-hexane. The resulting organic layer was washed with water and an aqueous sodium chloride solution in this order and then dried over anhydrous magnesium sulfate. The resulting solution was subjected to vacuum distillation to remove the solvent contained therein, to obtain 13.9 g (yield: 40.6%) of 3-chloro-5-ethyl-5-methyl-2-isoxazoline as a light yellow viscous liquid.

^{1}H-NMR [$CDCl_3$/TMSδ(ppm)]: 2.91 (2H, Abq, J=17.0, Δυ=46.1 Hz), 1.73 (2H,q), 1.42 (3H,s), 0.96 (3H,t)

Below are shown the properties (^{1}H-NMR [$CDCl_3$/TMSδ (ppm)]) of the present compounds produced according to the Production Process 1 or the Production Process 2.

Present compound 1-602: 6.71–7.23 (3H,m), 4.84 (2H,s), 4.04 (2H,q), 2.81 (2H,s), 2.47 (3H,s), 1.42 (6H,s)

Present compound 1-603: 6.72–7.23 (3H,m), 4.85 (2H,s), 3.93 (2H,t), 2.82 (2H,s), 2.47 (3H,s), 1.83 (2H,m), 1.42 (6H,s), 1.04 (3H,t)

Present compound 1-605: 6.72–7.29 (3H,m), 4.85 (2H,s), 3.98 (2H,t), 2.81 (2H,s), 2.47 (3H,s), 1.80 (2H,m), 1.38 (6H,s), 0.97 (3H,t)

Present compound 1-606: 6.72–7.27 (3H,m), 6.05 (1H,m), 5.43 (1H,d), 5.29 (1H,d), 4.87 (2H,s), 4.57 (2H,d), 2.88 (2H,s), 2.48 (3H,s), 1.44 (6H,s)

Present compound 1-607: 6.92–7.30 (3H,m), 4.84 (2H,s), 4.71 (2H,d), 2.96 (2H,s) 2.52 (1H,s), 2.48 (3H,s), 1.46 (6H,s)

Present compound 1-228: 7.44–7.34 (2H,min), 7.02–6.92 (2H,m), 4.71 (2H,s), 3.86 (3H,s), 2.81 (2H, ABq, J=117.4, Δυ=54.2 Hz), 1.68 (2H,q), 1.36 (3H,s), 0.90 (3H,t)

Present compound 1-590: 7.28 (1H,dd), 7.08 (1H,d), 6.86 (1H,d), 6.05 (1H,m), 5.45 (1H,d), 5.32 (1H,d), 4.90 (2H,s), 4.63 (2H,d), 3.00 (2H,s), 1.47 (6H,s)

Present compound 1-599: 8.07 (1H,d), 7.47–7.56 (3H,m), 6.05 (1H,m), 5.42 (1H,d), 5.31 (1H,d), 5.31 (2H,s), 4.83 (2H,d), 2.94 (2H,s) 1.43 (6H,s)

The herbicide of the present invention contains, as the active ingredient, an isoxazoline derivative represented by the genera formula [I] or a salt thereof.

In using the compound of the present invention as a herbicide, the present compound may be used by itself. It can also be used in the form of a powder, a wettable powder, an emulsifiable concentrate, fine granules, granules, etc. by blending with a carrier, a surfactant, a dispersant, an adjuvant, etc. all generally used in formulation.

As the carrier used in formulation, there can be mentioned, for example, solid carriers such as talc, bentonite, clay, kaolin, diatomaceous earth, white carbon, vermiculite, calcium carbonate, slaked lime, siliceous sand, ammonium sulfate, urea and the like; and liquid carriers such as isopropyl alcohol, xylene, cyclohexane, methylnaphthalene and the like.

As the surfactant and the dispersant, there can be mentioned, for example, metal salts of alkylbenzenesulfonic acids, metal salts of dinaphthylmethanedisulfonic acid, salts of alcohol sulfates, alkylarylsulfonic acid salts, ligninsulfonic acid salts, polyoxyethylene glycol ether, polyoxyethylene alkyl aryl ethers, monoalkylates of polyoxyethylene sorbitan and the like. As the adjuvant, there can be mentioned, for example, carboxymethyl cellulose, polyethylene glycol and gum arabic. The present herbicide, when used, is diluted to an appropriate concentration and sprayed or applied directly.

The herbicide of the present invention can be used by spraying on plant foliage, application to soil, application on water surface, etc. The amount of the active ingredient used is determined appropriately so as to meet the application purpose. When the present compound is made into a powder or granules, the amount is appropriately determined in a range of 0.01 to 10% by weight, preferably 0.05 to 5% by weight. When the present compound is made into an emulsifiable concentrate or a wettable powder, the amount is appropriately determined in a range of 1 to 90% by weight, 5 to 50% by weight.

The amount of the present herbicide used varies depending upon the kind of the compound used, the target weed, the tendency of weed emergence, the environmental conditions, the type of the herbicide used, etc. When the present herbicide is used per se as in the case of a powder or granules, the amount is appropriately selected in a range of 0.1 g to 5 kg, preferably 1 g to 1 kg per 10 ares in terms of the active ingredient. When the present herbicide is used in a liquid form as in the case of an emulsifiable concentrate or a wettable powder, the amount is appropriately selected in a range of 0.1 to 50,000 ppm, preferably 10 to 10,000 ppm in terms of the active ingredient.

The compound of the present invention may be mixed as necessary with an insecticide, a fungicide, other herbicide, a plant growth-regulating agent, a fertilizer, etc.

Next, formulation from the present compound is described specifically by showing typical examples of formulation. The kinds of compounds and additives and their compounding ratios are not restricted to those shown below and can be varied widely. In the following description, "parts" refer to parts by weight.

<Formulation 1>Wettable Powder

10 parts of a compound (1-5) are mixed with 0.5 part of polyoxyethylene octylphenyl ether, 0.5 part of a sodium salt of a β-naphthalenesulfonic acid-formalin condensate, 20 parts of diatomaceous earth and 69 parts of clay. The mixture is mixed and pulverized to obtain a wettable powder.

<Formulation 2>Wettable Powder

10 parts of a compound (1-5) are mixed with 0.5 part of polyoxyethylene octylphenyl ether, 0.5 part of a sodium salt of a β-naphthalenesulfonic acid-formalin condensate, 20 parts of diatomaceous earth, 5 parts of white carbon and 64 parts of clay. The mixture is mixed and pulverized to obtain a wettable powder.

<Formulation 3>Wettable Powder

10 parts of a compound (1-5) are mixed with 0.5 part of polyoxyethylene octylphenyl ether, 0.5 part of a sodium salt of a β-naphthalenesulfonic acid-formalin condensate, 20 parts of calcium carbonate. The mixture is mixed and pulverized to obtain a wettable powder.

<Formulation 4>Emulsifiable Concentrate

To 30 parts of a compound (1-5) are added 60 parts of an equal volume mixture of xylene and isophorone and 10 parts of a surfactant mixture of a polyoxyethylene sorbitan alkylate, a polyoxyethylene alkylaryl polymer and an alkylarylsulfonate. The resulting mixture is stirred sufficiently to obtain an emulsifiable concentrate.

<Formulation 5>Granules

There are mixed 10 parts of a compound (1-5), 80 parts of an extender which is a 1:3 mixture of talc and bentonite, 5 parts of white carbon, 5 parts of a surfactant mixture of a polyoxyethylene sorbitan alkylate, a polyoxyethylene alkylaryl polymer and an alkylarylsulfonate. The resulting mix-

ture is kneaded sufficiently to form a paste. The paste is extruded through the eyes (diameter: 0.7 mm) of a sieve. The extrudate is dried and cut into a length of 0.5 to 1 mm to obtain granules.

Next, Test Examples of the present compound are described to show the effect of the present compound.

<Test Example 1>

Test For Herbicidal Effect By Paddy Field Soil Treatment

A paddy field soil was filled in a plastic pot of 100 cm^2 and subjected to puddling. Then, seeds of *Echinochloa oryzicola Vasing*. (*Eo*) were sowed and water was filled in a depth of 3 cm. Next day, wettable powders produced in accordance with the Formulation 1 were diluted with water and dropped on the water surface. The application amount of each wettable powder was 100 g per 10 ares in terms of the active ingredient. Then, breeding was made in a greenhouse, and the herbicidal effect of each wettable powder was examined at the 21st day from the treatment in accordance with the standard shown in Table 40. The results are shown in Tables 41 to 43.

TABLE 40

Index	Herbicidal effect (extent of growth inhibition) or phytotoxicity
5	A herbicidal effect or phytotoxicity of 90% or more
4	A herbicidal effect or phytotoxicity of 70% to less than 90%
3	A herbicidal effect or phytotoxicity of 50% to less than 70%
2	A herbicidal effect or phytotoxicity of 30% to less than 50%
1	A herbicidal effect or phytotoxicity of 10% to less than 30%
0	A herbicidal effect or phytotoxicity of 0% to less than 10%

TABLE 41

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Eo
1-1	100	5
1-2	100	5
1-3	100	5
1-4	100	5
1-5	100	5
1-6	100	5
1-7	100	5
1-8	100	5
1-32	100	5
1-33	100	5
1-34	100	5
1-35	100	5
1-38	100	5
1-39	100	5
1-40	100	5
1-41	100	5
1-42	100	5
1-43	100	5
1-46	100	5
1-49	100	5
1-55	100	5
1-56	100	5
1-58	100	5
1-59	100	5
1-136	100	5

TABLE 41-continued

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Eo
1-139	100	5
1-142	100	5
1-145	100	5
1-157	100	5
1-158	100	5
1-160	100	5
1-184	100	5
1-185	100	5
1-186	100	5
1-187	100	5
1-188	100	5
1-189	100	5
1-190	100	5
1-191	100	5
1-192	100	5
1-193	100	5
1-194	100	5
1-195	100	5
1-198	100	5
1-199	100	5
1-200	100	5
1-201	100	5
1-202	100	5
1-203	100	5
1-228	100	5
1-229	100	5
1-230	100	5
1-328	100	5
1-329	100	5
1-331	100	5
1-336	100	5
1-363	100	5
1-364	100	5
1-365	100	5
1-366	100	5
1-367	100	5
1-368	100	5
1-377	100	5
1-378	100	5
1-380	100	5
1-381	100	5
1-382	100	5
1-383	100	5
1-384	100	5
1-386	100	4
1-387	100	5
1-388	100	5
1-394	100	5
1-396	100	5
1-397	100	5
1-401	100	5
1-419	100	5
1-456	100	5
1-457	100	5

TABLE 42

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Eo
1-487	100	5
1-488	100	5
1-489	100	5
1-490	100	5
1-491	100	5
1-492	100	5
1-493	100	5
1-494	100	5
1-495	100	5

TABLE 42-continued

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Eo
1-496	100	5
1-497	100	5
1-498	100	5
1-499	100	5
1-500	100	5
1-501	100	5
1-502	100	5
1-503	100	5
1-504	100	5
1-505	100	5
1-506	100	5
1-507	100	5
1-508	100	5
1-509	100	5
1-510	100	5
1-511	100	5
1-512	100	5
1-513	100	5
1-514	100	5
1-515	100	5
1-516	100	5
1-517	100	5
1-518	100	5
1-519	100	5
1-520	100	5
1-521	100	5
1-522	100	5
1-523	100	5
1-524	100	5
1-525	100	5
1-526	100	5
1-527	100	5
1-528	100	5
1-529	100	5
1-530	100	5
1-531	100	5
1-532	100	5
1-533	100	5
1-534	100	5
1-535	100	5
1-536	100	5
1-537	100	5
1-538	100	5
1-539	100	5
1-540	100	5
1-541	100	5
1-542	100	5
1-543	100	5
1-544	100	5
1-545	100	5
1-546	100	5
1-547	100	5
1-548	100	5
1-549	100	5
1-550	100	5
1-551	100	5
1-552	100	4
1-553	100	5
1-554	100	5
1-555	100	5
1-556	100	5
1-559	100	5
1-560	100	5
1-561	100	5
1-562	100	5
1-563	100	5
1-564	100	5
1-565	100	5
1-566	100	5
1-567	100	5
1-568	100	5

TABLE 43

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Eo
1-569	100	5
1-570	100	5
1-571	100	5
1-572	100	5
1-573	100	5
1-574	100	5
1-575	100	5
1-576	100	5
1-579	100	5
1-580	100	5
1-581	100	5
1-583	100	5
1-584	100	5
1-585	100	5
1-586	100	5
1-588	100	5
1-589	100	5
1-590	100	5
1-591	100	5
1-593	100	5
1-594	100	5
1-595	100	5
1-596	100	5
1-597	100	5
1-598	100	5
1-599	100	5
1-600	100	5
1-601	100	5
1-602	100	5
1-603	100	5
1-604	100	5
1-605	100	5
1-606	100	5
1-607	100	5
1-608	100	5
1-609	100	5
1-610	100	5
1-612	100	5
1-613	100	5
1-614	100	5
1-615	100	5
1-616	100	5
1-617	100	5
1-618	100	5
1-619	100	5
1-620	100	5
1-621	100	5
1-622	100	5
1-623	100	5
1-624	100	5
1-625	100	5
1-626	100	5
1-627	100	5
1-628	100	5
2-2	100	5
2-28	100	5
2-29	100	5

<Test Example 2>

Test For Herbicidal Effect By Upland Field Soil Treatment

An upland field soil was filed in a plastic pot of 80 cm^2. Seeds of *Echinochloa crus-galli (L.) Beauv.* var. *crusgall (Ec)* and *Setaria viridis (L.) Beauv.* (Se) were sowed, followed by covering with the same soil. Wettable powders produced in accordance with the Formulation 1 were diluted with water and sprayed uniformly: on the soil surface using a small sprayer, in an amount of 100 liters per 10 ares so that the amount of each active ingredient became 100 g per 10

ares. Then, breeding was made in a greenhouse, and the herbicidal effect of each wettable powder was examined at the 21st day from the treatment in accordance with the standard shown in Table 40. The results are shown in Tables 44 to 46.

TABLE 44

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Ec	Se
1-1	100	5	5
1-2	100	5	5
1-3	100	5	5
1-4	100	5	5
1-5	100	5	5
1-6	100	5	5
1-7	100	5	5
1-8	100	5	5
1-32	100	5	5
1-33	100	5	5
1-34	100	5	5
1-35	100	5	5
1-38	100	4	4
1-39	100	5	5
1-40	100	5	5
1-41	100	5	5
1-42	100	5	4
1-43	100	5	5
1-46	100	5	5
1-49	100	5	5
1-55	100	5	5
1-56	100	5	5
1-58	100	5	5
1-59	100	5	5
1-106	100	5	5
1-139	100	5	5
1-142	100	5	5
1-145	100	5	5
1-157	100	5	5
1-158	100	5	5
1-160	100	5	5
1-184	100	5	4
1-185	100	5	5
1-186	100	5	5
1-187	100	5	5
1-188	100	5	5
1-189	100	5	5
1-190	100	5	5
1-191	100	5	5
1-192	100	5	5
1-193	100	5	4
1-194	100	5	5
1-195	100	5	5
1-198	100	4	5
1-199	100	5	5
1-200	100	5	5
1-201	100	5	5
1-202	100	5	4
1-203	100	5	4
1-228	100	5	5
1-229	100	5	5
1-328	100	4	4
1-329	100	5	5
1-331	100	5	5
1-336	100	5	5
1-363	100	5	4
1-364	100	5	5
1-366	100	5	5
1-368	100	5	4
1-378	100	5	5
1-380	100	5	4
1-381	100	4	4
1-382	100	5	5
1-383	100	5	4
1-384	100	5	4
1-387	100	5	4

TABLE 44-continued

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Ec	Se
1-388	100	5	5
1-394	100	4	—
1-396	100	4	5
1-487	100	5	5
1-488	100	5	4
1-489	100	5	5
1-490	100	5	4
1-491	100	5	5
1-492	100	5	5
1-493	100	5	4
1-494	100	5	5
1-495	100	5	5
1-496	100	5	4
1-497	100	5	5

TABLE 45

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Ec	Se
1-498	100	5	5
1-499	100	5	5
1-500	100	5	5
1-501	100	5	5
1-502	100	5	5
1-503	100	5	5
1-504	100	5	5
1-505	100	5	5
1-506	100	5	5
1-507	100	5	5
1-508	100	5	5
1-509	100	5	5
1-510	100	5	5
1-511	100	5	5
1-512	100	5	5
1-513	100	5	5
1-514	100	5	5
1-515	100	5	5
1-516	100	5	5
1-517	100	5	5
1-518	100	5	4
1-520	100	5	5
1-521	100	5	5
1-522	100	5	5
1-523	100	5	5
1-524	100	5	5
1-525	100	5	5
1-526	100	5	5
1-527	100	5	5
1-528	100	5	5
1-529	100	5	5
1-530	100	5	5
1-531	100	5	5
1-532	100	5	4
1-534	100	5	5
1-535	100	5	5
1-536	100	5	5
1-537	100	5	5
1-538	100	5	5
1-539	100	5	5
1-540	100	5	5
1-541	100	5	5
1-542	100	5	5
1-543	100	5	5
1-544	100	5	5
1-545	100	5	5
1-546	100	5	4

TABLE 45-continued

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Ec	Herbicidal effect to Se
1-547	100	5	5
1-548	100	5	5
1-549	100	5	5
1-550	100	5	5
1-551	100	5	5
1-553	100	5	5
1-554	100	5	5
1-555	100	5	5
1-556	100	5	4
1-559	100	5	5
1-560	100	5	5
1-561	100	5	5
1-562	100	5	5
1-563	100	5	5
1-564	100	5	5
1-565	100	5	5
1-566	100	5	5
1-567	100	5	5
1-568	100	5	5
1-569	100	5	5
1-570	100	5	5
1-571	100	5	—
1-572	100	5	5
1-573	100	5	5
1-574	100	5	5
1-576	100	5	5
1-580	100	5	—
1-581	100	5	5
1-583	100	5	5
1-585	100	5	5
1-586	100	5	5
1-589	100	5	5
1-590	100	5	5

TABLE 46

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Ec	Herbicidal effect to Se
1-591	100	5	5
1-593	100	5	4
1-594	100	5	4
1-595	100	5	5
1-596	100	5	5
1-597	100	5	4
1-599	100	5	5
1-600	100	5	—
1-601	100	5	—
1-602	100	5	5
1-603	100	5	5
1-604	100	5	5
1-605	100	5	4
1-606	100	5	5
1-607	100	5	5
1-608	100	5	5
1-609	100	5	5
1-610	100	5	5
1-615	100	5	5
1-616	100	5	—
1-617	100	5	5
1-618	100	5	5
1-619	100	5	4
1-620	100	5	5
1-621	100	5	4
1-622	100	5	—
2-29	100	5	5

<Test Example 3>

Test For Herbicidal Effect By Upland Foliage Treatment

An upland field soil was filed in a plastic pot of 80 cm^2. Seeds of *Echinochlou crus-galli (L.) Beauv.* var. *crusgalli* (*Ec*) were sowed. Breeding was made in a greenhouse for 2 weeks. Wettable powders produced in accordance with the Formulation 1 were diluted with water and sprayed on the whole foliage of plants from above the plants using a small sprayer in an amount of 100 liters per 10 arcs so that the amount of each active ingredient became 100 g per 10 ares. Then, breeding was made in the greenhouse, and the herbicidal effect of each wettable powder was examined at the 14th day from the treatment in accordance with the standard shown in Table 40. The results are shown in Tables 47 and 48.

TABLE 47

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Ec
1-1	100	5
1-2	100	4
1-3	100	4
1-4	100	4
1-5	100	5
1-6	100	4
1-7	100	4
1-8	100	5
1-32	100	5
1-35	100	4
1-39	100	5
1-40	100	5
1-43	100	5
1-46	100	4
1-49	100	5
1-55	100	4
1-56	100	5
1-58	100	5
1-59	100	5
1-106	100	4
1-139	100	4
1-142	100	4
1-145	100	4
1-157	100	5
1-158	100	5
1-160	100	5
1-184	100	4
1-185	100	5
1-186	100	5
1-187	100	5
1-188	100	4
1-192	100	4
1-193	100	5
1-199	100	4
1-200	100	4
1-201	100	5
1-202	100	4
1-203	100	4
1-229	100	4
1-336	100	5
1-363	100	4
1-364	100	4
1-366	100	4
1-378	100	5
1-380	100	4
1-383	100	4
1-397	100	4
1-487	100	4
1-488	100	4
1-489	100	4
1-490	100	4
1-491	100	4

TABLE 47-continued

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Ec
1-492	100	4
1-494	100	5
1-495	100	5
1-496	100	5
1-497	100	5
1-498	100	5
1-499	100	5
1-500	100	5
1-501	100	5
1-502	100	5
1-503	100	4
1-504	100	5
1-505	100	5
1-506	100	4
1-507	100	4
1-508	100	4
1-509	100	4
1-510	100	4
1-511	100	5
1-512	100	5
1-513	100	5
1-514	100	5
1-515	100	5
1-516	100	5
1-517	100	5
1-520	100	4
1-521	100	5
1-522	100	5

TABLE 48

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Ec
1-523	100	5
1-524	100	5
1-525	100	5
1-526	100	5
1-527	100	5
1-528	100	5
1-529	100	5
1-530	100	5
1-531	100	5
1-532	100	4
1-534	100	5
1-535	100	4
1-536	100	4
1-537	100	4
1-538	100	5
1-539	100	5
1-540	100	5
1-541	100	4
1-542	100	4
1-543	100	4
1-544	100	5
1-545	100	5
1-547	100	5
1-548	100	5
1-550	100	5
1-553	100	5
1-554	100	5
1-555	100	4
1-556	100	4
1-559	100	5
1-560	100	4
1-561	100	4
1-562	100	4
1-563	100	4
1-564	100	4

TABLE 48-continued

Compound No.	Application amount (active ingredient), g/10 a	Herbicidal effect to Ec
1-565	100	4
1-566	100	4
1-567	100	4
1-568	100	5
1-569	100	5
1-570	100	5
1-571	100	5
1-572	100	4
1-573	100	5
1-574	100	4
1-576	100	5
1-581	100	4
1-583	100	4
1-584	100	5
1-585	100	5
1-586	100	5
1-589	100	4
1-590	100	4
1-591	100	4
1-593	100	4
1-595	100	4
1-599	100	4
1-602	100	4
1-603	100	4
1-604	100	5
1-606	100	4
1-607	100	5
1-608	100	5
1-609	100	5
1-616	100	5

<Test Example 4>

Test For Crop Selectivity By Paddy Field Soil Treatment

A paddy field soil was filled in a plastic pot of 100 cm^2 and subjected to puddling. Seeds of Echinochloa oryzicola Vasing. (Eo) were sowed; two-leaf stage seedlings of rice (Or) were transplanted in a depth of 2 cm; and water was filled in a depth of 3 cm. Next day, wettable powders produced in accordance with the Formulation 1 were diluted with water and dropped on the water surface. The application amount of each wettable powder was 100 g per 10 ares in terms of the active ingredient. Then, breeding was made in a greenhouse. At the 21st day from the treatment, the phytotoxicity and herbicidal effect of each wettable powder were examined in accordance with the standard shown in Table 40. The results are shown in Table 49.

TABLE 49

Compound No.	Application amount of active ingredient g/10 a	Phytotoxicity to Or	Herbicidal effect to Es
1-145	100	0	5
1-190	100	1	5
1-198	100	1	5
1-203	100	1	5
1-228	100	1	5
1-229	100	1	5
1-230	100	0	5
1-328	100	0	5
1-331	100	0	5
1-365	100	1	5

TABLE 49-continued

Compound No.	Application amount of active ingredient: g/10 a	Phytotoxicity to Oh	Herbicidal effect to Ec
1-367	100	1	5
1-368	100	1	5
1-377	100	0	5
1-384	100	0	5
1-386	100	0	4
1-394	100	1	5
1-401	100	0	5
1-419	100	1	5
1-456	100	0	5
1-457	100	1	5
1-503	100	0	5
1-518	100	1	5
1-519	100	0	5
1-520	100	1	5
1-549	100	1	5
1-552	100	1	4
1-556	100	0	5
1-574	100	1	5
1-579	100	1	5
1 580	100	1	5
1 584	100	1	5
1-588	100	1	5
1-590	100	1	5
1-594	100	1	5
1-596	100	1	5
1-597	100	1	5
1-598	100	1	5
1-599	100	1	5
1-601	100	1	5
1-605	100	1	5
1-610	100	1	5
1-612	100	0	5
1-613	100	0	5
1-614	100	0	5
1-615	100	1	5
1-620	100	1	5
1-622	100	1	5
1-623	100	1	5
1-624	100	1	5
1-625	100	1	5
1-626	100	0	5
1-627	100	1	5
1-628	100	0	5
2-2	100	0	5
2-29	100	1	5

TABLE 50

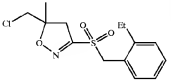
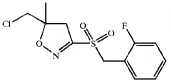
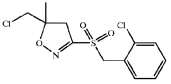
Compound	Structural formula	Patent No. and Compound No.
Comparative compound 1		JP-A-8-225548 and 2012
Comparative compound 2		JP-A-8-225548 and 2059
Comparative compound 3		JP-A-8-225548 and 2034

TABLE 51

Compound No.	Application amount of active ingredient g/10 a	Herbicidal effect to Mo	Herbicidal effect to Sc
1-8	25	5	4
1-39	25	5	5
1-49	25	5	5
1-58	25	4	5
1-157	25	5	5
1-382	25	5	4
1-547	25	5	5
1-597	25	5	5
Comparative compound 1	25	1	1
Comparative compound 2	25	1	1

<Test Example 5>

Test For Herbicidal Effect During Breeding Period By Paddy Field Water Treatment

A paddy field soil was filled in a plastic pot of 100 cm^2 and subjected to puddling. Seeds of *Monochoria vaginalis Presl* (*Mo*) and *Scirpus juncoides Roxb.* subsp. *juncoides Roxb.* (*Sc*) were sowed; water was filled in a depth of 3 cm; and breeding was made. When *Mo* reached a 1-leaf stage and Sc reached a 2-leaf stage, wettable powders produced in accordance with the Formulation 1 were diluted with water and dropped on the water surface. The application amount of each wettable powder was 100 g per 10 ares in terms of the active ingredient. Then, breeding was made in a greenhouse. At the 30th day from the treatment, the herbicidal effect of each wettable powder was examined in accordance with the standard shown in Table 40. The results are shown in Table 51. Incidentally, the details of comparative compounds 1 and 2 are shown in Table 50.

<Test Example 6>

Test for herbicidal effect to broadleaf weeds by upland field soil treatment

An upland field soil was filled in a plastic pot of 80 cm^2. Seeds of *Polygonum lapathifolium L.* subsp. *nodosum* (*Pers.*) *Kitam.* (*Po*) and *Chenopodium album L.* (*Ch*) were sowed, followed by covering with the same soil. Wettable powders produced in accordance with the Formulation 1 were diluted with water and sprayed uniformly on the soil surface using a small sprayer, in an amount of 100 liters per 10 ares so that the amount of each active ingredient became 100 g per 10 ares. Then, breeding was made in a greenhouse. At the 30th day from the treatment, the herbicidal effect of each wettable powder was examined in accordance with the standard shown in Table 40. The results are shown in Table 52. Incidentally, the details of comparative compounds 1 and 3 are shown in Table 50.

TABLE 52

Compound	Application amount of active ingredient	Herbicidal effect to	
No.	g/10 a	Po	Ch
1-2	25	—	4
1-39	25	4	5
1-46	25	5	4
1-498	25	5	5
1-499	25	5	4
1-500	25	5	4
1-501	25	5	5
1-523	25	5	5
1-526	25	5	5
1-532	25	5	5
1-534	25	5	5
1-555	25	5	4
1-573	25	5	5
Comparative 1	25	0	0
Comparative 3	25	1	0

Industrial Applicability

The compound represented by the general formula [I] according to the present invention shows an excellent herbicidal effect, at a low application amount over a wide period, from before germination to growth, to various weeds causing problems in upland fields, for example, Gramineae weeds [e.g. *Echinochloa crus-galli* (*L.*) *Beauv.* var. *crus-galli*, *Digitaria ciliaris* (*Retz.*) *Koeler*, *Setaria viridis* (*L.*) *Beauv*, *Poa annua L.*, *Sorghum halepense* (*L.*) *Pers.*, *Alopecurus aequalis Sobol.* var. *amurensis* (*Komar.*) *Ohwi*, and wild oats], broadleaf weeds [*Polygonum lapathifolium L. nodosum* (*Pers.*) *Kitam.*, *Amaranthus viridis L.*, *Chenopodium album L.*, *Stellaria media* (*L.*) Villars, *Abutilon avicennae*, *Sida spinosa*, *cassia obtusifolia*, *Ambrosia artemisiifolia L.* var. *elatior* (*L.*) Desc., and morning glory], and perennial or annual cyperaceous weeds [e.g. *Cyperus rotundus L.*, *cyperus esculentus*, *Kyllinga brevifolia Rottb.* subsp. *leiolepis* (*Fraxch.* et *Savat.*) *T. Koyama*, *Cyperus microiria Steud.*, and *Cyperus iria L.*].

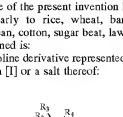
Further, the present compound shows a herbicidal effect, at a low application amount over a wide period from before germination to growth, also to weeds emerging in paddy fields, i.e. annual weeds [e.g. *Echinochloa oryzicola Vasing.*, *Cyperus difformis L.*, *Monochoria vaginalis* (*Burm. f.*) *Presl.* var. *plantaginea* (*Roxb.*) *Solms-Laub.*, and *Lindernia pyxidara L.*] and perennial weeds [e.g. *Cyperus serotinus Rottb.*, *Eleocharis kuroguwai Ohwi*, and *Scirpus juncoides Roxb.* subsp. *hotarui* (*Ohwi*) *T. Koyama*].

The herbicide of the present invention has high safety to crops, particularly to rice, wheat, barley, corn, grain sorghum, soybean, cotton, sugar beat, lawn, fruit trees, etc.

What is claimed is:

1. An isoxazoline derivative represented by the following general formula [I] or a salt thereof:

[I]



wherein Q is a group represented by $—S(O)_n—(CR_5R_6)_m$- (wherein n is 0 or an integer of 1 to 2, m is an integer of 1 to 3, and R_5 and R_6 are each independently a hydrogen atom, a cyano group, an alkoxycarbonyl group or a C_1 to C^6 alkyl group);

R_1 and R_2 are each independently a hydrogen atom, a C_1 to C_8 alkyl group [which may be substituted with C_3 to C_8 cycloalkyl group, C_1 to C_6 alkoxy group, C_1 to C_6 alkylcarbonyl group, C_1 to C_6 alkylthio group, C_1 to C_6 alkylsulfinyl group, C_1 to C_6 alkylsulfonyl group, C_1 to C_6 alkylamino group, di(C_1 to C_6 alkyl)amino group, cyano group, C_1 to C_6 alkoxycarbonyl group, C_1 to C_6 alkylaminocarbonyl group, di(C_1 to C_6 alkyl) aminocarbonyl group, (C_1 to C_6 alkylthio)carbonyl group, carboxyl group, optionally substituted benzyloxy group, optionally substituted phenoxy group, or optionally substituted phenyl group], a C_3 to C_8 cycloalkyl group, a C_1 to C_6 alkoxycarbonyl group, a C_3 to C_8 alkylaminocarbonyl group, a di (C_1 to C_6 alkyl) aminocarbonyl group, a C_1 to C_6 alkylthiocarbonyl group, a carboxyl group or an optionally substituted phenyl group;

R_3 and R_4 are each independently a hydrogen atom, a C_1 to C_8 alkyl group (which may be substituted with 1 to 3 same or different, C_3 to C_8 cycloalkyl groups or C_1 to C_6 alkoxy groups) or a C_3 to C_8 cycloalkyl group; and

Y is a phenyl group substituted with 1 to 5 same or different R_9; each R_9 is a hydrogen atom, a C_1 to C_6 alkyl group [which may be substituted with 1 to 3 same or different halogen atoms, C_1 to C_6 alkoxy groups, hydroxyl groups, C_1 to C_6 alkylthio groups, C_1 to C_6 alkylsulfinyl groups, C_1 to C_6 alkylsulfonyl groups, C_1 to C_6 alkylamino groups, di(C_1 to C_6)alkylamino groups, cyano groups or optionally substituted phenoxy groups], a C_1 to C_6 alkoxy group (which may be substituted with 1 to 3 same or different halogen atoms, C_1 to C_6 alkoxy groups, C_2 to C_6 alkenyl groups, C_1 to C_6 alkynyl groups, C_1 to C_6 alkoxycarbonyl groups, C_1 to C_6 alkylcarbonyl groups or C_3 to C_8 cycloalkyl groups), a C_2 to C_6 alkenyl group, a C_2 to C_6 cycloalkyloxy group, a C_1 to C_6 alkylthio group (which may be substituted with 1 to 3 same or different halogen atoms or C_1 to C_6 alkoxy groups), a C_1 to C_6 alkylsulfinyl group (which may be substituted with 1 to 3 same or different halogen atoms or C_1 to C_6 alkoxy groups), a C_1 to C_6 alkylsulfonyl group (which may be substituted with 1 to 3 same or different halogen atoms or C_1 to C_6 alkoxy groups), an optionally substituted benzyloxy group, an amino group [which may be substituted with C_1 to C_6 alkyl group, C_1 to C_6 alkylsulfonyl group, C_1 to C_6 alkylcarbonyl(C_1 to C_6 alkyl) group or C_1 to C_6 alkylsulfonyl (C_1 to C_6 alkyl) group], a di(C_1 to C_6 alkyl)amino group, a halogen atom, a cyano group, a nitro group, a C_1 to C_6 alkoxycarbonyl group, a C_4 to C_9 cycloalkyloxycarbonyl group, a carboxyl group, a C_2 to C_6 alkenyloxycarbonyl group, a C_2 to C_6 alkynyloxycarbonyl group, an optionally substituted benzyloxycarbonyl group, an optionally substituted phenoxycarboriyl group or a C_7 to C_8 alkylcarbonyloxy group; provided that compounds wherein R_1, R_2, R_3 and R_4 are hydrogen at the same time are excluded.

2. An isoxazoline derivative or a salt thereof according to claim **1**, wherein in the general formula [I],

Q is a group represented by $—S(O)_n—(CR_5R_6)_m$- (wherein n is 0 or an integer of 1 to 2, m is 1, and R_5 and R_6 are a hydrogen atom);

R_1 and R_2 are each independently a hydrogen atom, a C_1 to C_8 alkyl group (which may be substituted with C_3 to C_6 cycloalkyl group or C_1 to C_6 alkoxy group) or a C_3 to C_8 cycloalkyl group;

R_3 and R_4 are each independently a hydrogen atom or a C_1 to C_8 alkyl group (which may be substituted with 1 to 3 same or different, C_3 to C_8 cycloalkyl groups or C_1 to C_8 alkoxy groups); and

Y is a phenyl group substituted with 1 to 5 same or different R_7; each R_7 is a hydrogen atom, a C_1 to C_6 alkyl group [which may be substituted with 1 to 3 same or different halogen atoms, C_1 to C_6 alkoxy groups, hydroxyl groups, C_1 to C_6 alkylthio groups, C_1 to C_6 alkylsulfinyl groups, C_1 to C_6 alkylsulfonyl groups, C_1 to C_6 alkylamino groups, C_1 to C_6 dialkylamino groups, cyano groups or optionally substituted phenoxy groups], a C_1 to C_6 alkoxy group (which may be substituted with 1 to 3 same or different halogen atoms, C_1 to C_6 alkoxy groups, C_2 to C_6 alkenyl groups, C_2 to C_6 alkynyl groups, C_1 to C_6 alkoxycarbonyl groups, C_1 to C_6 alkylcarbonyl groups or C_3 to C_8 cycloalkyl groups), a C_3 to C_8 cycloalkyloxy group or a halogen atom; provided that compounds wherein R_1, R_2, R_3 and R_4 are hydrogen at the same time are excluded.

3. An isoxazoline derivative or a salt thereof according to claim **1**, wherein in the general formula [I],

Q is a group represented by $-S(O)_n-(CR_8R_9)_m-$ (wherein n is 0 or an integer of 1 to 2, m is 1, and R_8 and R_9 are a hydrogen atom);

R_1 and R_2 are a C_1 to C_8 alkyl group;

R_3 and R_4 are a hydrogen atom;

Y is a phenyl group substituted with 1 to 5 same or different R_7; each R_7 is a hydrogen atom, a C_1 to C_6 alkyl group (which may be substituted with 1 to 3 same or different halogen atoms or C_1 to C_6 alkoxy groups), a C_1 to C_6 alkoxy group (which may be substituted with 1 to 3 same or different halogen atoms or C_1 to C_6 alkoxy groups), or a halogen atom.

4. A herbicidal formulation containing, as the active ingredient, an isoxazoline derivative or its salt set forth in claim **1**.

5. A herbicidal formulation containing, as the active ingredient, an isoxazoline derivative or its salt set forth in claim **2**.

6. A herbicidal formulation containing, as the active ingredient, an isoxazoline derivative or its salt set forth in claim **3**.

7. An isoxazoline compound or a salt according to claim **1**, wherein m is 1; and

R_1 and R_2 are each independently a hydrogen atom, a C_1 to C_8 alkyl group [which may be substituted with C_1 to C_H cycloalkyl group, C_1 to C_6 alkoxy group, C_1 to C_6 alkylcarbonyl group, C_1 to C_6 alkylthio group, C_1 to C_6 alkylsulfinyl group, C_1 to C_6 alkylsulfonyl group, C_1 to C_6 alkylamino group, di(C_1 to C_6 alkyl)amino group, cyano group, C_1 to C_6 alkoxycarbonyl group, C_1 to C_6 alkylaminocarbonyl group, di(C_1 to C_6 alkyl) aminocarbonyl group, (C_1 to C_6 alkylthio)carbonyl group, carboxyl group, optionally substituted benzyloxy group, optionally substituted phenoxy group, or optionally substituted phenyl group], a C_3 to C_8 cycloalkyl group, a C_1 to C_6 alkoxycarbonyl group, a C_1 to C_6 alkylaminocarbonyl group, a di(C_1 to C_6 alkyl)aminocarbonyl group, a (C_1 to C_6 alkylthio) carbonyl group, a carboxyl group or an, optionally substituted phenyl group.

8. An isoxazoline compound or a salt according to claim **1**, wherein m is 1;

R_5 and R_6 are each independently a hydrogen atom or a C_1 to C_6 alkyl group;

R_1 and R_2 are each independently a hydrogen atom, a C_1 to C_H alkyl group [which may be substituted with C_3 to C_6 cycloalkyl group or C_1 to C_6 alkoxy group], or a C_3 to C_8 cycloalkyl group; and

R_3 and R_4 are each independently a hydrogen atom or a C_1 to C_8 alkyl group [which may be substituted with 1 to 3 same or different, C_3 to C_8 cycloalkyl group or C_1 to C_6 alkoxy group].

9. An isoxazoline compound or a salt according to claim **1**, wherein m is 1;

R_5 and R_6 are each independently a hydrogen atom or a C_1 to C_6 alkyl group;

R_1 and R_2 are each independently a hydrogen atom, a C_1 to C_8 alkyl group which may be substituted with C_3 to C_8 cycloalkyl group, or a C_3 to C_8 cycloalkyl group; and

R_3 and R_4 are each independently a hydrogen atom or a C_1 to C_8 alkyl group.

10. An isoxazoline compound or a salt according to claim **1**, wherein m is 1;

R_5 and R_6 are each independently a hydrogen atom or a C_1 to C_6 alkyl group;

R_1 and R_2 are each independently a hydrogen atom, a C_1 to C_8 alkyl group which may be substituted with C_3 to C_8 cycloalkyl group, or a C_3 to C_8 cycloalkyl group;

R_3 and R_4 are each independently a hydrogen atom or a C_1 to C_8 alkyl group; and

Y is a hydrogen atom, a C_1 to C_{10} alkyl group which may be substituted with 1 to 3 same or different halogen atoms, or a phenyl group substituted with 1 to 5 same or different R_7; R_7 is a hydrogen atom, a C_1 to C_6 alkyl group which may be substituted with 1 to 3 same or different halogen atoms, a C_1 to C_6 alkoxy group [which may be substituted with 1 to 3 same or different halogen atoms, C_2 to C_6 alkenyl group, C_2 to C_6 alkynyl group, or C_3 to C_6 cycloalkyl group], a C_3 to C_8 cycloalkyloxy group, a C_1 to C_6 alkylthio group [which may be substituted with 1 to 3 same or different halogen atoms or C_1 to C_6 alkoxy group], a halogen atom, a cyano group, a nitro group, a C_1 to C_6 alkoxycarbonyl group, or a C_3 to C_8 cycloalkyloxycarbonyl group.

11. An isoxazoline compound or a salt according to claim **1**, wherein

Q is a group represented by $-S(O)_n-(CR_8R_9)_m-$ (wherein n is 2, m is 1 and R_8 and R_9 are a hydrogen atom);

R_1 and R_2 are each independently a C_1 to C_3 alkyl group;

R_3 and R_4 are a hydrogen atom; and

Y is a phenyl group substituted with 1 to 5 same or different

R_7; R_7 is a hydrogen atom, a C_1 to C_6 alkyl group which may be substituted with 1 to 3 same or different halogen atoms or a C_1 to C_6 alkoxy group, a C_1 to C_6 alkoxy group, a halogen atom or a halogen atom.

12. An isoxazoline compound or a salt according to claim **1**, wherein

Q is a group represented by $-S(O)_n-(CR_8R_9)_m-$ (wherein n is 2, m is 1 and R_8 and R_9 are a hydrogen atom);

R_1 and R_2 are a methyl group;

R_3 and R_4 are a hydrogen atom; and

Y is a phenyl group substituted with 1 to 5 same or different R_7; R_7 is a hydrogen atom, a C_1 to C_6 alkyl

group which may be substituted with 1 to 3 same or different halogen atoms, a C_1 to C_6 alkoxy group which may be substituted with 1 to 3 same or different halogen atoms, or a halogen atom.

13. An isoxazoline compound or a salt according to claim **1**, wherein

Q is a group represented by $—S(O)_n—(CR_8R_9)_m—$ (wherein n is 2, m is 1 and R5 and R6 are a hydrogen atom);

R_1 and R_2 are a methyl group;

R_3 and R_4 are a hydrogen atom; and

Y is a phenyl group substituted with 1 to 5 same or different R_7; R_7 is a hydrogen atom, a C_1 to C_3 alkyl group which may be substituted with 1 to 3 same or different halogen atoms, a C_1 to C_3 alkoxy group which may be substituted with 1 to 3 same or different halogen atoms, or a halogen atom.

14. An isoxazoline compound or a salt according to claim **1**, wherein

n is 2, m is 1 and R_8 and R_9 are a hydrogen atom;

R_1 and R_2 are each independently a C_1 to C_2 alkyl group; and

Y is a phenyl group substituted with 1 to 5 same or different R_7; R_7 is a hydrogen atom, a halogen atom, a C_1 to C_3 alkyl group, a C_1 to C_3 alkoxy group, a C_1 to C_3 haloalkyl group or a C_1 to C_3 haloalkoxy group.

15. An isoxazoline compound or a salt according to claim **14**, wherein

R_1 and R_2 are a methyl group; and

R_7 is a is a hydrogen atom, F, Cl, Br, a C_1 to C_3 alkyl group, a C_1 to C_3 alkoxy group, a C_1 to C_3 fluoroalkyl group, a C_1 to C_3 chloroalkyl group, a C_1 to C_3 fluoroalkoxy group or a C_1 to C_3 chloroalkoxy group.

16. A herbicidal formulation containing, as the active ingredient, an isoxazoline derivative or its salt set forth in claim **7**.

17. A herbicidal formulation containing, as the active ingredient, an isoxazoline derivative or its salt set forth in claim **8**.

18. A herbicidal formulation containing, as the active ingredient, an isoxazoline derivative or its salt set forth in claim **9**.

19. A herbicidal formulation containing, as the active ingredient, an isoxazoline derivative or its salt set forth in claim **10**.

20. A herbicidal formulation containing, as the active ingredient, an isoxazoline derivative or its salt set forth in claim **11**.

21. A herbicidal formulation containing, as the active ingredient, an isoxazoline derivative or its salt set forth in claim **12**.

22. A herbicidal formulation containing, as the active ingredient, an isoxazoline derivative or its salt set forth in claim **13**.

23. A herbicidal formulation containing, as the active ingredient, an isoxazoline derivative or its salt set forth in claim **14**.

24. A herbicidal formulation containing, as the active ingredient, an isoxazoline derivative or its salt set forth in claim **15**.

25. An isoxazoline compound or salt according to claim **1**, wherein

Q is a group represented by $—S(O)_n—(CR_8R_9)_m—$ (wherein n is 2, m is 1 and R_8 and R_9 are a hydrogen atom);

R_1 and R_2 are methyl group;

R_3 and R_4 are a hydrogen atom; and

Y is a phenyl group substituted with 1 to 5 same or different R_7; R_7 is a hydrogen atom, a chlorine atom or a C_1 to C_3 alkoxy group.

26. An isoxazoline compound or salt according to claim **1**, wherein

Q is a group represented by $—S(O)_n—(CR_8R_9)_m—$ (wherein n is 2, m is 1 and R_8 and R_9 are a hydrogen atom);

R_1 and R_2 are methyl group;

R_3 and R_4 are a hydrogen atom; and

Y is a phenyl group substituted with 1 to 5 same or different R_7; R_7 is a hydrogen atom, a chlorine atom or a C_1 to C_3 fluoroalkoxy group.

27. An isoxazoline compound or salt according to claim **1**, wherein

Q is a group represented by $—S(O)_n—(CR_8R_9)_m—$ (wherein n is 2, m is 1 and R_8 and R_9 are a hydrogen atom);

R_1 and R_2 are methyl group;

R_3 and R_4 are a hydrogen atom; and

Y is a phenyl group substituted with 1 to 5 same or different R_7; R_7 is a hydrogen atom, a chlorine atom or a 2-propynyloxy group.

* * * * *

Exhibit D

US006743814B2

(12) **United States Patent**
Watanabe et al.

(10) **Patent No.: US 6,743,814 B2**
(45) **Date of Patent: Jun. 1, 2004**

(54) **NEMATICIDAL TRIFLUOROBUTENES**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(52) **U.S. Cl.** **514/376**; 548/229; 548/230

(58) **Field of Search** 548/229, 230; 514/376

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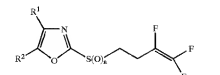
(74) *Attorney, Agent, or Firm*—Richard E. L. Henderson

(57) **ABSTRACT**

The present invention relates to novel trifluorobutenes of the formula (I)



(I)

wherein

R^1 represents hydrogen; halogen; alkyl that is unsubstituted or substituted with halogen, hydroxy, alkoxy, alkylthio, alkylcarbonyloxy, haloalkylcarbonyloxy, or cyano; alkylsulfonyloxy; or phenyl that is unsubstituted or substituted with halogen, alkyl, haloalkyl, alkoxy, alkylthio, alkylsulfonyl, haloalkoxy, haloalkylthio, phenyl, phenoxy, cyano, or nitro;

R^2 represents hydrogen; halogen; alkyl that is unsubstituted or substituted with alkoxy or halogen; or alkoxycarbouyl; and

n represents 0, 1 or 2,

with the proviso that if R^1 represents alkyl, then R^2 does not represent halogen. The invention also relates to processes for their preparation and their use as nematicides.

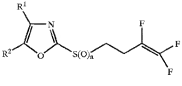
8 Claims, No Drawings

NEMATICIDAL TRIFLUOROBUTENES

The present invention relates to novel trifluorobutenes, processes for their preparation and their use as a nematicidal agent.

Japanese Laid-open Patent Publication No. 85267/1990 describes substituted azolethioethers which have insecticidal activity. U.S. Pat. No. 3,513,172 describes that some kinds of trifluorobutenyl compounds have nematicidal activity and Japanese Laid-open Patent Publication (PCT) No. 500037/1988 describes that some kinds of polyhaloalkene compounds have nematicidal activities. Further, WO 95/24403 describes that 4,4-difluorobutenyl compounds have nematicidal activity.

There have now been found novel trifluorobutenes of formula (I)



(I)

wherein

R^1 represents hydrogen, halogen, or alkyl which may be unsubstituted or substituted with halogen, hydroxy, alkoxy, alkylthio, alkylcarbonyloxy, haloalkylcarbonyloxy or cyano, or represents alkylsulfonyloxy or represents phenyl which may be unsubstituted or substituted with halogen, alkyl, haloalkyl, alkoxy, alkylthio, alkylsulfonyl, haloalkoxy, haloalkylthio, phenyl, phenoxy, cyano or nitro.

R^2 represents hydrogen, halogen, or alkyl which may be unsubstituted or substituted with alkoxy or halogen, or represents alkoxycarbonyl, and

n represents 0, 1 or 2.

provided that if R^1 represents alkyl, R^2 does not represent halogen.

In the definitions, the hydrocarbon chains, such as alkyl, are in each case straight-chain or branched. Substituents may be identical or different.

Preferred substituents or preferred ranges of the radicals present in the formulae listed above and below are defined below.

R^1 preferably represents hydrogen or halogen, or represents C_{1-6} alkyl which may be unsubstituted or substituted with halogen, hydroxy, C_{1-3} alkoxy, C_{1-3} alkylthio, C_{1-3} alkylcarbonyloxy, trifluoromethylcarbonyloxy or cyano, or represents C_{1-4} alkylsulfonyloxy or represents phenyl which may be unsubstituted or substituted with halogen, methyl, trifluoromethyl, methoxy, methylthio, methylsulfonyl, trifluoromethoxy, trifluoromethylthio, phenyl, phenoxy, cyano or nitro.

R^2 preferably represents hydrogen or halogen or represents C_{1-6} alkyl which may be unsubstituted or substituted with C_{1-3} alkoxy or halogen, or represents C_{1-4} alkoxycarbonyl.

n preferably represents 0 or 2.

R^1 particularly preferably represents hydrogen, fluoro, chloro or bromo, or represents C_{1-4} alkyl which may be unsubstituted or substituted with fluoro, chloro, bromo, hydroxy, C_{1-3} alkoxy, C_{1-3} alkoxy, C_{1-3} alkylthio, C_{1-3} alkylcarbonyloxy, trifluoromethylcarbonyloxy or cyano, or represents methylsulfonyloxy or ethylsulfonyloxy or represents phenyl which may be unsubstituted or substituted with fluoro, chloro, bromo, methyl, trifluoromethyl, methoxy, methylthio, methylsulfonyl, trifluoromethoxy, trifluoromethylthio, phenyl, phenoxy, cyano or nitro.

R^2 particularly preferably represents hydrogen, fluoro, chloro or bromo or represents C_{1-4} alkyl which may be unsubstituted or substituted with C_{1-3} alkoxy or bromo or represents C_{1-3} alkoxycarbonyl.

n particularly preferably represents 0.

The novel compounds of the above-mentioned formula (I) are obtained, for example, by the following preparation processes a), b), c), d), e), f), g) or h).

Preparation Process a)

Compounds of the formula (I)

wherein

R^1 represents hydrogen, represents alkyl which may be substituted with halogen, hydroxy, alkoxy, alkylthio, alkylcarbonyloxy, haloalkylcarbonyloxy or cyano or represents phenyl which may be substituted with halogen, alkyl, haloalkyl, alkoxy, alkylthio, alkylsulfonyl, haloalkoxy, haloalkylthio, phenyl, phenoxy, cyano or nitro,

R^2 represents hydrogen or represents alkyl which may be substituted with alkoxy or halogen and

n represents 0

are obtained when compounds of the formula (II)

(II)

wherein

R^{1a} represents hydrogen, represents alkyl which may be substituted with halogen, hydroxy, alkoxy, alkylthio, alkylcarbonyloxy, haloalkylcarbonyloxy or cyano or represents phenyl which may be substituted with halogen, alkyl, haloalkyl, alkoxy, alkylthio, alkylsulfonyl, haloalkoxy, haloalkylthio, phenyl, phenoxy, cyano or nitro,

R^{2a} represents hydrogen or represents alkyl which may be substituted with alkoxy or halogen and

n represents 0

are reacted with 4-bromo-1,1,2-trifluoro-1-butene in the presence of inert solvents and if appropriate, in the presence of an acid binder.

Preparation Process b)

Compounds of the formula (I)

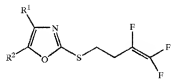
wherein

R^1 and R^2 are as defined above, and

n represents 1 or 2

are obtained when compounds of the formula (Ia)

(Ia)



wherein

R^1 and R^2 are as defined above

are oxidized in the presence of inert solvents.

Preparation Process c)

Compounds of the formula (I)

wherein

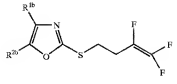
R^1 represents hydrogen or haloalkyl,

R^2 represents halogen, and

n represents 0

are obtained when compounds of the formula (Ib)

(Ib)



wherein

R^{1b} represents hydrogen or alkyl, and

R^{2b} represents hydrogen

are reacted with a halogenating agent in the presence of inert solvents.

Preparation Process d)

Compounds of the formula (I)

wherein

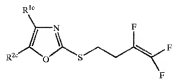
R^1 represents haloalkyl,

R^2 represents hydrogen or haloalkyl and

n represents 0

are obtained when compounds of the formula (Ic)

(Ic)



wherein

R^{1c} represents alkyl, and

R^{2c} represents hydrogen or alkyl,

are reacted with a halogenating agent in the presence of inert solvents.

Preparation Process e)

Compounds of the formula (I)

wherein

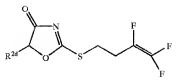
R^1 represents halogen,

R^2 represents hydrogen or alkyl and

n represents 0,

are obtained when compounds of the formula (III)

(III)



wherein

R^{2d} represents hydrogen or alkyl,

are reacted with a halogenating agent in the presence of inert solvents, and, if appropriate, in the presence of an organic base.

Preparation Process f)

Compounds of the formula (I)

wherein

R^1 represents alkylsulfonyloxy,

R^2 represents hydrogen or alkyl and

n represents 0

are obtained when compounds of the aforementioned formula (III) are reacted with compounds of the formula

$$R^{1d}SO_2Cl \quad \text{(IV)}$$

wherein

R^{1d} represents alkyl

in the presence of inert solvents, and if appropriate, in the presence of an inorganic or organic base.

Preparation Process g)

Compounds of the formula (I)

wherein

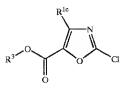
R^1 represents alkyl,

R^2 represents alkoxycarbonyl and

n represents 0

are obtained when compounds of the formula (IV)

(V)



wherein

R^{1c} is as defined above, and

R^3 represents alkyl,

are reacted with thiourea and the products are then reacted with 4-bromo-1,1,2-trifluoro-1-butene in the presence of inert solvents.

Preparation Process h)

Compounds of the formula (I)

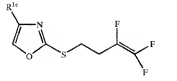
wherein

R^1 represents hydrogen,

R^2 represents alkoxyalkyl and

n represents 0

are obtained when compounds of the formula (Id)



(Id)

wherein

R^{1e} represents hydrogen,

are reacted with compounds of the formula (VI)

(VI)

wherein

R^4 represents alkoxyalkyl,

in the presence of inert solvents.

The compounds of the formula (I) of the present invention exhibit strong nematicidal activity and show good compatibility with various crops.

The compounds of the formula (I) according to the present invention surprisingly show a very outstanding nematicidal activity compared with the compounds described in the aforementioned literature which are similar to the compounds of the present invention.

In the present specification "halogen" represents fluoro, chloro, bromo or iodo, preferably represents fluoro, chloro or bromo, and particularly preferably represents chloro or bromo.

"Alkyl" and each alkyl part of "alkoxy", "alkylthio", "alkylcarbonyloxy", "alkylsulfonyloxy" and "alkoxycarbonyl" represents a straight-chain or branched-chain alkyl such as methyl, ethyl, n- or i-propyl, n-, i-, s- or t-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, preferably represents methyl, ethyl, n- or i-propyl, n-, i-, s-, or t-butyl, n-pentyl or n-hexyl and particularly preferably represents methyl, ethyl, n- or i-propyl or n-, i-, s-, or t-butyl.

"Haloalkyl" and each haloalkyl part of "haloalkylcarbonyloxy", "haloalkoxy" and "haloalkylthio" represents alkyl substituted with at least one halogen, preferably represents C_{1-4} alkyl substituted with one or a plurality of halogen, and particularly preferably represents methyl, ethyl or n- or i-propyl substituted with one or a plurality of fluoro, chloro or bromo. "Haloalkyl" preferably represents chloromethyl, bromomethyl or trifluoromethyl.

Very particular emphasis is given to the group of the compounds of the formula (I) wherein

R^1 represents hydrogen, halogen, represents methyl, ethyl, n- or i-propyl, n-, i-, s- or t-butyl, n-pentyl or n-hexyl, each of which may be substituted with halogen, hydroxy, methoxy, ethoxy, n- or i-propoxy, methylthio, ethylthio, n- or i-propylthio, methylcarbonyloxy, ethylcarbonyloxy, n- or i-propylcarbonyloxy, trifluoromethylcarbonyloxy or cyano, or represents methylsulfonyloxy, ethylsulfonyloxy, n- or i-propylsulfonyloxy, n-, i-, s- or t-butylsulfonyloxy or represents phenyl which may be substituted with halogen, methyl, trifluoromethyl, methoxy, methylthio, methylsulfonyl, trifluoromethoxy, trifluoromethylthio, phenyl, phenoxy, cyano or nitro,

R^2 represents hydrogen, halogen, methyl, ethyl, n- or i-propyl, n-, i-, s-, or t-butyl, n-pentyl or n-hexyl each of which may be substituted with methoxy, ethoxy, n- or i-propoxy or halogen or represents methoxycarbonyl, ethoxycarbonyl, n- or i-propoxycarbonyl or n-, i-, s-, or t-butoxycarbonyl, and

n represents 0, 1 or 2.

However, if R^1 represents methyl, ethyl, n- or i-propyl, n-, i-, s- or t-butyl, n-pentyl or n-hexyl, R^2 does not represent halogen.

Very particular preference is furthermore given to the group of compounds of the formula (I), wherein

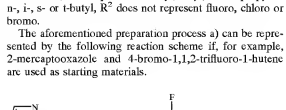
R^1 represents hydrogen, fluoro, chloro, bromo, represents methyl, ethyl, n- or i-propyl, n-, i-, s- or t-butyl, each of which may be substituted with fluoro, chloro, bromo, hydroxy, methoxy, ethoxy, n- or i-propoxy, methylthio, ethylthio, n- or i-propylthio, trifluoromethylcarbonyloxy or cyano, or represents methylsulfonyloxy or ethylsulfonyloxy, or represents phenyl which may be substituted with fluoro, chloro, bromo, methyl, trifluoromethyl, methoxy, methylthio, methylsulfonyl, trifluoromethoxy, trifluoromethylthio, phenyl, phenoxy, cyano or nitro,

R^2 represents hydrogen, fluoro, chloro, bromo, represents methyl, ethyl, n- or i-propyl or n-, i-, s- or t-butyl each of which may be substituted with methoxy, ethoxy, n- or i-propoxy or hromo or represents methoxycarbonyl, ethoxycarbonyl or n- or i-propoxycarbonyl, and

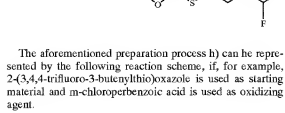
n represents 0, 1 or 2.

However, if R^1 represents methyl, ethyl, n- or i-propyl or n-, i-, s- or t-butyl, R^2 does not represent fluoro, chloro or bromo.

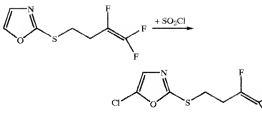
The aforementioned preparation process a) can be represented by the following reaction scheme if, for example, 2-mercaptooxazole and 4-bromo-1,1,2-trifluoro-1-butene are used as starting materials.



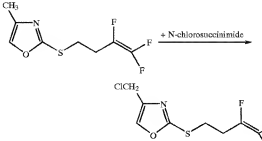
The aforementioned preparation process h) can he represented by the following reaction scheme, if, for example, 2-(3,4,4-trifluoro-3-butenylthio)oxazole is used as starting material and m-chloroperbenzoic acid is used as oxidizing agent.



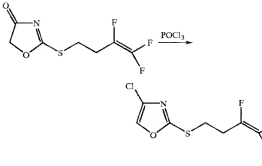
The aforementioned preparation process c) can be represented by the following reaction scheme, if, for example, 2-(3,4,4-trifluoro-3-butenylthio)oxazole is used as starting material and sulfuryl chloride is used as halogenating agent.



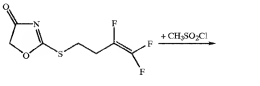
The aforementioned preparation process d) can be represented by the following reaction scheme, if, for example, 4-methyl-2-(3,4,4-trifluoro-3-butenylthio)oxazole is used as starting material and N-chlorosuccinimide is used as halogenating agent.



The aforementioned preparation process e) can be represented by the following reaction scheme, if, for example, 2-(3,4,4-trifluoro-3-butenylthio)oxazolidin-4-one is used as starting material and phosphorus oxychloride is used as halogenating agent.



The aforementioned preparation process f) can be represented by the following reaction scheme, if, for example, 2-(3,4,4-trifluoro-3-butenylthio)oxazolidin-4-one and methanesulfonic chloride are used as starting materials.



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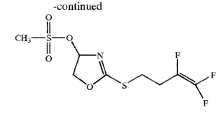
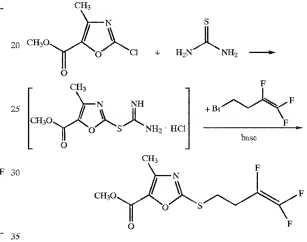
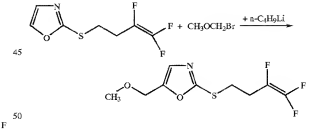


The aforementioned preparation process g) can bc represented by the following reaction scheme, if, for example, 2-chloro-5-methoxycarbonyl-4-methyloxazole, thiourea and 4-bromo-1,1,2-trifluoro-1-butene are used as starting materials.



The aforementioned preparation process h) can be represented by the following reaction scheme, if, for example, 2-(3,4,4-trifluoro-3-butenylthio)oxazole and bromomethyl methyl ether are used as starting materials:



The compounds of the formula (II), used as starting material in the aforementioned preparation process a) include the known compounds described in the literature, for example in J. Org. Chem., (1988), 53 (5), 1113–1114; Collect. Czech. Chem. Commun. (1983), 48 (12), 3421–3425; Can. J. Chem., (1972), 50 (18), 3082–3083 etc.

As specific examples of the compounds of the formula (II) there can be mentioned,

2-mercaptooxazole,
2-mercapto-4-methyloxazole,
4-ethyl-2-mercaptooxazole,
2-mercapto-5-methyloxazole,
5-ethyl-2-mercaptooxazole,
2-mercapto-5-n-propyloxazole,
2-mercapto-4-n-propyloxazole,

2-mercapto-4-iso-propyloxazole,
2-mercapto-4-tert-butyloxazole,
2-mercapto-4,5-dimethyloxazole,
2-mercapto-4-phenyloxazole and so on.

4-Bromo-1,1,2-trifluoro-1-butene, used as starting material in the aforementioned preparation process a), is a known compound described in the document WO 86/07590.

The compounds of the formula (Ia), used as starting material in the aforementioned preparation process b), correspond to the compounds of formula (I) if n represents 0 and can be synthesized, for example, according to the aforementioned preparation process a).

As oxidizing agent used for the oxidation of the compounds of the above-mentioned formula (Ia) in the preparation process b) there can be mentioned those which are used usually in the field of organic chemistry, for example, hydrogen peroxide water, m-chloroperbenzoic acid, peracetic acid, perbenzoic acid, magnesium monoperoxyphthalate, potassium peroxymonosulfate and so on.

The compounds of the formula (Ib) and the formula (Ic), used as starting materials in the aforementioned preparation process c) and preparation d), respectively, are generally described by the formula (I) of the present invention, wherein n represents 0. They can be synthesized, for example, according to the aforementioned preparation process a).

As halogenating agent, which can be reacted with the compounds of the formula (Ib) and the formula (Ic) in the preparation process c) and preparation d), respectively, there can be mentioned those which are used usually in the field of organic chemistry, for example, sulfuryl chloride, N-chlorosuccinimide, N-bromosuccinimide, trichloroisocyanuric acid, potassium fluoride, chlorine gas, bromine, iodine and so on.

The compounds of the formula (III) used as starting materials in the aforementioned preparation process e) and preparation f) are novel compounds which were not described in the literature before. They can be prepared, for example, according to the following process i).

Preparation Process i)

Compounds of the formula (III) are obtained when compounds of the formula (VII)

(VII)

wherein

$R^{2,4}$ is as defined before,

are reacted with 4-bromo-1,1,2-trifluoro-1-butene in the presence of an inert solvent, and, if appropriate, in the presence of an acid binder.

The compounds of the above-mentioned formula (VII) include known compounds and can be synthesized, for example, according to the process described in Ukrain. Khim. Zhur., 16, 545–551 (1950).

As specific examples of the compounds of the aforementioned formula (III) there can be mentioned, for example,

2-(3,4,4-trifluoro-3-butenylthio)oxazolidin-4-one,

5-methyl-2-(3,4,4-trifluoro-3-butenylthio)oxazolidin-4-one and so on.

As halogenating agents, which can be reacted with the compounds of the aforementioned formula (III) in the preparation process e) there can be mentioned phosphorus oxychloride, phosphorus oxybromide, phosphorus pentachloride and so on.

The compounds of the aforementioned formula (IV), which are needed in the preparation process f), are well known in the field of organic chemistry. Specific examples which can be mentioned are methanesulfonic chloride, ethanesulfonic chloride etc.

The compounds of the formula (V) used as starting materials in the aforementioned preparation process g), are known compounds and can be prepared, for example, according to the process described in document WO 95/24403.

Specific examples of the compounds of the aforementioned formula (V) which can be mentioned are 2-chloro-5-methoxycarbonyl-4-methyloxazole, 2-chloro-5-ethoxycarbonyl-4-methyloxazole etc.

The compounds of the formula (Id) used as starting materials in the preparation process h) are generally described by the formula (I) of the present invention and can be synthesized, for example, according to the aforementioned preparation process a). Further, the compounds of the formula (VI), which are needed in the preparation process h), are well known compounds in the field of organic chemistry. Specific examples which can be mentioned are bromomethyl methyl ether, bromomethyl ethyl ether etc.

The reaction of the preparation process a) can be conducted in the presence of an adequate diluent. Examples of the diluents which can be used here are aliphatic, alicyclic and aromatic hydrocarbons, such as hexane, cyclohexane, petroleum ether, ligroine, benzene, toluene or xylene; ethers, such as, diethyl ether, methyl ethyl ether, di-isopropyl ether, dibutyl ether, propylene oxide, dioxane or tetrahydrofuran; ketones, such as acetone, methyl ethyl ketone or methyl isobuiyl ketone; nitriles, such as acetonitrile, propionitrile or acrylonitrile; acid amides, such as, dimethylformamide, dimethylacetamide or N-methylpyrrolidone.

The reaction of the preparation process (a) can be conducted in the presence of an acid binder. Acid binders which can be used are, for example, hydroxides, carbonates and alcoholates etc. of alkali metals, tertiary amines, such as, triethylamine, diethylaniline, pyridine, 4-dimethylaminopyridine, 1,4-diazabicyclo[2,2,2]octane (DABCO) or 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU).

The reaction of the preparation process a) can be conducted in a substantially wide range of temperature. In general, the processes are carried out at temperatures between 0° C. and 150° C., preferably between 20° C. and 100° C. Although said reaction is generally carried out under normal pressure, it can be optionally carried out under elevated pressure or under reduced pressure.

When carrying out the preparation process a), the compounds of the corresponding formula (I) can be obtained by reacting, for example, 0.7–1.5 moles of 4-bromo-1,1,2-trifluoro-1-butene with 1 mole of the compounds of the formula (II) in a diluent, for example, acetonitrile in the presence of 1–1.3 moles of an acid binder, for example, potassium carbonate, under reflux by heating.

Among the compounds of the formula (I) of the present invention which can be prepared by the preparation process a), the compounds the formula (I) wherein R^1 represents hydroxymethyl, alkoxymethyl, halogenomethyl, alkylcarbonyloxy, alkylthiomethyl or cyanomethyl, R^2 represents hydrogen and n represents 0, can be also synthesized according to other processes described in Synthesis Examples 10–14 below.

The reaction of the preparation process b) can be carried out in the presence of an adequate diluent. Examples of the

diluents which can be used are aliphatic, alicyclic and aromatic hydrocarbons which may be optionally chlorinated, such as, hexane, cyclohexane, petroleum ether, ligroine, benzene, toluene, xylene, methylene chloride, chloroform, carbon tetrachloride, ethylene chloride or chlorobenzene; ethers, such as, diethyl ether, methyl ethyl ether, di-isopropyl ether, dibutyl ether, propylene oxide, dioxane or tetrahydrofuran; alcohols, such as, methanol, ethanol, isopropanol, butanol or ethylene glycol; esters, such as, ethyl acetate or amyl acetate; acid amides, such as, dimethylformamide, dimethylacetamide or N-methylpyrrolidone; carboxylic acids, such as, formic acid or acetic acid.

The reaction of the preparation process b) can be conducted in a substantially wide range of temperatures. In general, the processes are carried out at temperatures between −20° C. and 100° C., preferably between 0° C. and 80° C. Although said reaction is generally carried out under normal pressure, it can be optionally carried out under elevated pressure or under reduced pressure.

When carrying out the preparation process b), the compounds of the corresponding formula (I) can be obtained by reacting, for example, 0.8–3 moles of m-chloroperbenzoic acid with 1 mole of the compounds of the formula (Ia) in a diluent, for example, methylene chloride, at room temperature.

The reaction of the preparation processes c) and d) can be carried out in the presence of an adequate diluent. Examples of the diluents which can be used are aliphatic, alicyclic and aromatic hydrocarbons which may be optionally chlorinated, such as, hexane, cyclohexane, petroleum ether, ligroine, benzene, toluene, xylene, methylene chloride, chloroform, carbon tetrachloride, ethylene chloride or chlorobenzene; ethers, such as, diethyl ether, methyl ethyl ether, di-isopropyl ether, dibutyl ether, propylene oxide, dioxane or tetrahydrofuran; acid amides, such as, dimethylformamide, dimethylacetamide or N-methylpyrrolidone; sulfones and sulfoxides, such as, dimethyl sulfoxide or sulfolane.

The reaction of the preparation processes c) and d) can be conducted in a substantially wide range of temperatures. In general, the processes are carried out at temperatures between −20° C. and 200° C., preferably between 0° C. and 150° C. Although said reaction is generally carried out under normal pressure, it can be optionally carried out under elevated pressure or under reduced pressure.

When carrying out the preparation processes c) and d), the compounds of the corresponding formula (I) can be obtained by reacting e.g. 1–4 moles of N-chlorosuccinimide with 1 mole of the compounds of the formula (Ib) in a diluent, such as, carbon tetrachloride, under reflux by heating.

The reaction of the preparation process e) can be carried out in the presence of an adequate diluent. Examples of the diluent which can be used are hydrocarbons including halogenated hydrocarbons, ethers, nitrites and acid amides according to the list of diluents mentioned in the aforementioned preparation process b).

The reaction of the preparation process (e) can be carried out in the presence of an organic base. Organic bases which can be used are, for example, triethylamine, 1,1,4,4-tetramethylethylenediamine (TMEDA), N,N-dimethylaniline or pyridine.

The reaction of the preparation process e) can be carried out in a substantially wide range of temperatures. In general, the processes are carried out at temperatures between 0° C. and 200° C., preferably between 20° C. and 120° C. Although said reaction is generally carried out under normal pressure, it can be optionally carried out under elevated pressure or under reduced pressure.

When carrying out the preparation process e), the compounds of the corresponding formula (I) can be obtained by reacting, for example, 1–5 moles of a halogenating agent to 1 mole of the compounds of the formula (III) in the presence of pyridine.

The reaction of the preparation process f) can be carried out in the presence of an adequate diluent. Examples of the diluents which can be used are the same diluents as mentioned in the aforementioned preparation process c) and, in addition, there can be mentioned alkylsulfonyl chlorides, for example, methanesulfonyl chloride, ethanesulfonyl chloride or isopropylsulfonyl chloride.

Further, the reaction of the preparation process (f) can be carried out in the presence of inorganic bases and organic bases. Organic bases which can be used are the same ones as exemplified in the aforementioned preparation process e). Inorganic bases which can be used are, for example, sodium carbonate or potassium carbonate.

The reaction of the preparation process f) can be carried out in a substantially wide range of temperature. In general, the processes are carried out at temperatures between −20° C. and 150° C., preferably between 0° C. and 130° C. Although said reaction is generally carried out under normal pressure, it can be optionally carried out under elevated pressure or under reduced pressure.

When carrying out the preparation process f), the compounds of the corresponding formula (I) can be obtained by reacting, for example, 1–3 moles of a compound of the formula (IV) to 1 mole of the compounds of the formula (III) with an organic base, for example triethylamine, in the presence of a diluent, for example tetrahydrofuran.

The reaction of the preparation process g) can be carried out in the presence of an adequate diluent. Diluents which can be used are the same diluents as mentioned in the aforementioned preparation process a) and, in addition, alcohols, for example, methanol, ethanol or isopropanol.

The reaction of the preparation process g) can be carried out in a substantially wide range of temperature. In general, the processes are carried out at temperatures between 0° C. and 150° C., preferably between 20° C. and 120° C. Although said reaction is generally carried out under normal pressure, it can be optionally carried out under elevated pressure or under reduced pressure.

When carrying out the preparation process g), the compounds of the corresponding formula (I) can be obtained by reacting, for example, 1–1.5 moles of thiourea to 1 mole of the compounds of the formula (V) in a diluent, for example, ethanol and then reacting with 1–1.5 moles of 4-bromo-1,1,2-trifluoro-1-butene.

The reaction of the preparation process h) can be carried out in the presence of an adequate diluent. Diluents which can be used are, for example, the ethers exemplified before.

The reaction of the preparation process h) can be carried out in a substantially wide range of temperature. In general, the processes are carried out at temperatures between 100° C. and 150° C., preferably between −78° C. and 100° C. Although said reaction is generally carried out under normal pressure, it can be optionally carried out under elevated pressure or under reduced pressure.

When carrying out the preparation process h), the compounds of the corresponding formula (I) can be obtained by reacting, for example, 1–1.5 moles of the compounds of the formula (VI) to 1 mole of the compound of the formula (Id) in a diluent, for example, diethyl ether, in the presence of n-butyl lithium.

The reaction of the preparation process i) can be carried out in the presence of an adequate diluent. Diluents which can be used are the same diluents as mentioned in the aforementioned preparation process a).

The reaction of the preparation process (i) can be carried out in the presence of the same acid binder as mentioned in the aforementioned preparation process a).

The reaction of the preparation process (i) can be carried out by applying the same reaction temperatures and pressures mentioned in the aforementioned preparation process a).

When carrying out the preparation process i), the compounds of the corresponding formula (III) can be obtained by reacting, for example, 0.7–1.5 moles of 4-bromo-1,1,2-trifluoro-1-butene to 1 mole of the compounds of the formula (VII) in a diluent, for example, tetrahydrofuran, in the presence of 1–1.3 moles of triethylamine.

The compounds of the formula (I) of the present invention show a strong ability to control nematodes. They can, therefore, be efficiently used as nematicidal agents.

Furthermore, the compounds of the formula (I) of the present invention show no phytotoxicity against crops and at the same time exhibit the ability to specifically control harmful nematodes.

Examples of nematodes against which the active compounds of the formula (I) of the present invention can be applied are, for example, Pratylenchus spp., *Globodera rostochiensis* Wollenweber, Heterodera spp., such as, *Heterodera glycines* Ichinohe, Meloidogyne spp., *Aphelenchoides basseyi* Christie, *Bursaphelenchus Xylophilis, Radiophotus similis, Ditylenchus dipsaci, Tylenchulus semipenetrans* etc. However, the nematodes which can be controlled by said compounds are not limited to the above list.

The active compounds of the present invention can also be used as mixtures with other active compounds, such as, insecticides, bactericides, miticides, fungicides etc. in the form of their commercially useful formulation or in the application form prepared from those formulations. Possible components for the mixtures are insecticides, for example organophosphorus agents, carbamate agents, carboxylate type chemicals, chlorinated hydrocarbon type chemicals or chloronicotinyl type chemicals, insecticidal substances produced by microbes.

Further, the active compounds of the present invention can also be used as mixtures with synergists in such formulations and application forms as can be mentioned as commercially useful. A synergist itself must not be active, but enhances the action of the active compound.

The content of the active compounds of the present invention in a commercially useful formulation or application form can be varied in a wide range. The application concentration of the active compounds of the formula (I) of the present invention can be in the range of generally 0.000001–100% by weight, preferably 0.00001–1% by weight.

The active compounds of the present invention can be converted into the customary formulations, such as, solutions, emulsions, wettable powders, water dispersible granules, suspensions, powders, foaming agents, pastes, granules, active compound-impregnated natural and synthetic substances, microcapsules, fumigants etc.

These formulations can be prepared according to per se known methods, for example by mixing the active compounds with extenders, namely liquid solvents, liquefied gas and/or solid diluents or carriers, and optionally with surfactants, namely emulsifiers and/or dispersants and/or foam formers. When water is used as extender, it is also possible to use, for example, organic solvents as auxiliary solvents.

Liquid diluents or carriers which can be used are, for example, aromatic hydrocarbons, such as, xylene, toluene or alkylnaphthalene, chlorinated aromatic or chlorinated aliphatic hydrocarbons, such as, chlorobenzenes, ethylene chlorides or methylene chloride, aliphatic hydrocarbons such as cyclohexane or paraffins, such as mineral oil fractions, alcohols, such as, butanol, glycols and their ethers or esters, ketones, such as, acetone, methyl ethyl ketone, methyl isobutyl ketone or cyclohexanone, strongly polar solvents, such as, dimethylformamide or dimethyl sulphoxide, water and so on.

Liquefied gas diluents or carriers are liquefied substances which are gases at normal temperature and pressure. Examples are aerosol propellants such as butane, propane, nitrogen gas, carbon dioxide and halogenated hydrocarbons.

Solid diluents which can be used are, for example, ground natural minerals such as kaolin, clay, talc, chalk, quartz, attapulgite, montmorillonite or diatomaceous earth, ground synthetic minerals such as highly dispersed silicic acid, alumina or silicates and so on.

Solid carriers for granules which can be used are for, example, crushed and fractionated rocks, such as, calcite, marble, pumice, sepiolite or dolomite synthetic granules of inorganic and organic meals, particles of organic materials, such as, sawdust, coconut shells, maize cobs or tobacco stalks and so on.

Emulsifiers and/or foam-forming agents which can be used are, for example, nonionic and aniontic emulsifiers, such as, polyoxyethylene fatty acid esters or polyoxyethylene fatty acid alcohol ethers, such as, alkylaryl polyglycol ethers, alkylsulphonates, alkylsulphates or arylsulphonates, albumin hydrolysis products and so on.

Dispersants include, for example, ligninsulphite waste liquor and methyl cellulose.

Tackifiers may also be used in formulations, such as, powders, granules or emulsions. Tackifiers which can be used are, for example, carboxymethyl cellulose, natural and synthetic polymers, such as, gum arabic, polyvinyl alcohol or polyvinyl acetate.

Colorants may also be used. Colorants which can be used are, for example, inorganic pigments such as iron oxide, titanium oxide or Prussian Blue, organic dyestuffs, such as, alizarin dyestuffs, azo dyestuffs or metal phthalocyanine dyestuffs, and further trace nutrients, such as, salts of metals such as iron, manganese, boron, copper, cobalt, molybdenum or zinc.

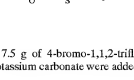
Said formulations generally comprise the aforementioned active components in a range of between 0.1–95% by weight, preferably between 0.5–90% by weight.

The preparation and use of the compounds of the present invention will be described more specifically in the following examples. However, the present invention should not be restricted to them in any way. "Parts" means "parts by weight" unless specified.

EXAMPLES

Preparation Examples

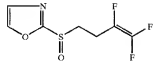
Example 1



7.5 g of 4-bromo-1,1,2-trifluoro-1-butene and 7.0 g of potassium carbonate were added to 50 ml of acetonitrile, 3.8

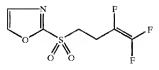
g of 2-mercaptooxazole, and refluxed for 5 hours by heating. After cooling the reaction mixture to room temperature, solid substance was filtered by suction and the filtrate was distilled off under reduced pressure. The residue was purified by column chromatography (eluent: hexane:dichloromethane=1:1) to obtain 6.5 g of 2-(3,4,4-trifluoro-3-butenylthio)oxazole as colorless oil (yield 82.7%, n^{20}_D=1.4631).

Example 2



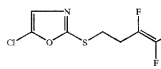
1.0 g of 2-(3,4,4-trifluoro-3-butenylthio)oxazole and 1.5 g of m-chloroperbenzoic acid (purity about 70%) were added to 50 ml of dichloromethane and stirred at room temperature for 20 hours. The reaction mixture was washed with 50 ml of 1N aqueous solution of sodium hydroxide and dried with magnesium sulfate. After the solvent was distilled off under reduced pressure, the residue was purified by column chromatography (eluent: ethyl acetate:dichloromethane=1:4) to obtain 1.0 g of 2-(3,4,4-trifluoro-3-butenylsulfinyl)oxazole as colorless oil (yield 92.9%, n^{20}_D=1.4820).

Example 3



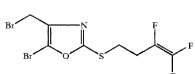
1.0 g of 2-(3,4,4-trifluoro-3-butenylthio)oxazole and 3.0 g of m-chloroperbenzoic acid (purity about 70%) were added to 50 ml of dichloromethane and stirred at room temperature for 20 hours. The reaction mixture was washed with 50 ml of 1N aqueous solution of sodium hydroxide and dried with magnesium sulfate. After the solvent was distilled off under reduced pressure, the residue was purified by column chromatography (eluent: dichloromethane) to obtain 0.8 g of 2-(3,4,4-trifluoro-3-butenylsulfonyl)oxazole as colorless oil (yield 69.4%, n^{20}_D=1.4705).

Example 4



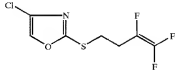
4.0 g of 2-(3,4,4-trifluoro-3-butenylthio)oxazole was added to 40 ml of DMF (dehydrated) to which a mixture of 10 ml of chloroform and 2.6 g of sulfuryl chloride was added dropwise at 50 in 1 hour. After the reaction mixture was stirred at 50° C. for 3 hours, a mixture of 5 ml of chloroform and 0.8 g of sulfuryl chloride was further added dropwise in 15 minutes. After stirring at 50° C. for 15 hours, the reaction mixture was cooled to room temperature, poured into 200 ml of ice water and extracted with 100 ml of hexane. The aqueous layer was neutralized with 1N aqueous solution of sodium hydroxide and further extracted with 100 ml of hexane. The extracted hexane layers were put together, washed with 200 ml of saturated common salt water and dried with magnesium sulfate. After the solvent was distilled off under reduced pressure, the residue was purified by column chromatography (eluent: hexane:dichloromethane=1:1) to obtain 2.4 g of 5-chloro-2-(3,4,4-trifluoro-3-butenylthio)oxazole as colorless oil (yield 51.5%, n^{20}_D=1.4830).

Example 5



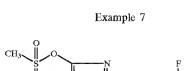
2.2 g of 4-methyl-2-(3,4,4-trifluoro-3-butenylthio) oxazole was dissolved in 40 ml of dichloromethane and 3.4 g of N-bromosuccinimide were added and stirred at room temperature for 5 hours. After the solvent was distilled off under reduced pressure, the residue was purified by column chromatography (eluent: hexane:ethyl acetate=93:7) to obtain 2.4 g of 5-bromo-4-bromomethyl-2-(3,4,4-trifluoro-3-butenylthio)oxazole (yield 63%). n^{20}_D=1.4963.

Example 6



2 g of phosphorus oxychloride were added dropwise to a mixture of 1 g of 2-(3,4,4-trifluoro-3-butenylthio) oxazolidin-4-one and 0.35 g of pyridine under ice cooling and stirred at 70–80° C. for 3 hours. After addition of ice water to the reaction mixture and stirring for 30 minutes, the mixture was extracted with dichloromethane. The extracted layer was washed with water, dried with anhydrous sodium sulfate and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography (eluent: dichloromethane) to obtain 0.7 g of 4-chloro-2-(3,4,4-trifluoro-3-butenylthio)oxazole (yield 65%). n^{20}_D=1.4813.

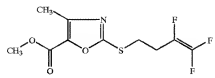
Example 7



1 g of 2-(3,4,4-trifluoro-3-butenylthio)oxazolidin-4-one and 0.9 g of triethylamine were dissloved in 30 ml of tetrahydrofuran, to which 0.56 g of methanesulfonic chloride was added dropwise under ice cooling and stirred at 50° C. for 8 hours. After distilling off the solvent under reduced pressure, the residue was dissolved in ether. After washing with 1N hydrochloric acid and water, it was dried with

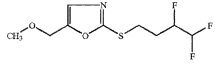
anhydrous sodium sulfate and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography (eluent: hexane:dichloromethane=1:1) to obtain 0.25 g of 4-methylsulfonyloxy-2-(3,4,4-trifluoro-3-butenylthio)oxazole (yield 19%). n^{20}_D=1.4830.

Example 8



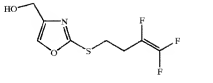
2.1 g of 2-chloro-5-methoxycarbonyl-4-methyloxazole and 1.1 g of thiourea were dissloved in 55 ml of ethanol and refluxed for 8 hours. After cooling, the solvent was distilled off under reduced pressure. The residue was dissolved in 55 ml of acetone, to which 2.5 g of 4-bromo-1,1,2-trifluoro-1-butene and 2.4 g of potassium carbonate were added, stirred at room temperature for 18 hours and the solvent was distilled off under reduced pressure. After addition of 50 ml of water to the residue and extraction with ether, the ether layer was washed with water and dried with anhydrous sodium sulfate. After the solvent was distilled off under reduced pressure, the residue was treated by silica gel column chromatography to obtain 3.1 g of 5-methoxycarbonyl-4-methyl-2-(3,4,4-trifluoro-3-butenylthio)oxazole. n^{20}_D=1.4972.

Example 9



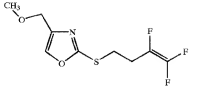
10.40 ml of n-butyl lithium (1.6 M n-hexane solution) were slowly added dropwise to a solution of 3.14 g of 2-(3,4,4-trifluoro-3-butenylthio)oxazole in 40 ml of ether at −70° C. After stirring for 1 hour, a solution of 2.06 g bromomethyl methyl ether in 10 ml ether was added dropwise. After bringing the mixture back to room temperature and stirring for 1 hour, 50 ml of saturated solution of ammonium chloride was added to separate it into an ether layer and an aqueous layer. The aqueous layer was further extracted with ether and together with the separated ether layer it was washed with a saturated sodium chloride water and dried with anhydrous magnesium sulfate. After the solvent was distilled off under reduced pressure, the residue was treated by column chromatography to obtain 0.5 g of 5-methoxymethyl-2-(3,4,4-trifluoro-3-butenylthio)oxazole (yield 13%). n^{25}_D=1.4705.

Example 10



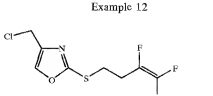
7.5 g of 3,8-dioxa-1,6-diazaspiro[4,4]nona-1,6-diene-2,7-dithiol, 7.5 g of 4-bromo-1,1,2-trifluoro-1-butene and 7.0 g of potassium carbonate were added to 200 ml of acetonitrile and refluxed for 5 hours. The reaction mixture was cooled to room temperature and filtered by suction and the filtrate was distilled under reduced pressure. The residue was purified by column chromatography (eluent: ethyl acetate:dichloromethane=1:4) to obtain 7.0 g of 4-hydroxymethyl-2-(3,4,4-trifluoro-3-butenylthio)oxazole as colorless oil (yield 74.2%). n^{20}_D=1.4910.

Example 11



A solution of 2.7 g of 4-hydroxymethyl-2-(3,4,4-trifluoro-3-butenylthio)oxazole in 50 ml of tetrahydrofuran (dehydrated) was added dropwise to a suspension of 0.5 g NaH (purity about 60%) in 20 ml of tetrahydrofuran (dehydrated) at 0° C. After stirring the mixture at room temperature for 1 hour, a solution of 2.0 g of methyl iodide in 20 ml of tetrahydrofuran (dehydrated) was added at 0° C. and further stirred at room temperature for 1 hour. The reaction mixture was poured into a mixed solution of 200 g ice and 2N hydrochloric acid. After extraction with 100 ml of dichloromethane, the solution was washed with water and dried with anhydrous magnesium sulfate. After the solvent was distilled off under reduced pressure, the residue was purified by column chromatography (eluent: dichloromethane) to obtain 1.5 g of 4-methoxymethyl-2-(3,4,4-trifluoro-3-butenylthio)oxazole as colorless oil (yield 52.5%). n^{20}_D=1.4710.

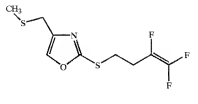
Example 12



6.8 g of 4-hydroxymethyl-2-(3,4,4-trifluoro-3-butenylthio)oxazole and 2.5 g of pyridine were dissolved in 30 ml of chloroform, to which a solution of 5.0 g of thionyl chloride in 10 ml of chloroform was added dropwise at 0° C. After the addition the mixture was refluxed for 10 hours. After cooling to room temperature, the solvent was distilled under reduced pressure. The residue was purified by column

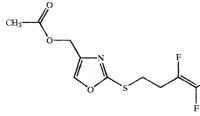
chromatography (eluent: hexane:dichloromethane=2:1) to obtain 5.7 g of 4-chloromethyl-2-(3,4,4-trifluoro-3-butenylthio)oxazole as colorless oil (yield 77.8%). n^{20}_D=1.4933.

Example 13



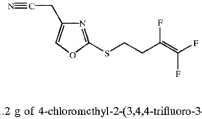
1.2 g of 4-chloromethyl-2-(3,4,4-trifluoro-3-butenylthio) oxazole, 0.5 g of sodium thiomethoxide and 0.1 g of sodium iodide were added to 20 ml of dimethylformamide (dehydrated) and stirred at 80° C. for 10 hours. After cooling, 200 ml of water were added to the reaction mixture and extracted with 100 ml of diethyl ether twice. The extracted diethyl ether layers were put together, washed with 200 ml of water, dried with magnesium sulfate and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography (eluent: hexane:dichloromethane=2:1) to obtain 0.3 g of 4-methylthiomethyl-2-(3,4,4-trifluoro-3-butenylthio) oxazole as colorless oil (yield 23.9%). n^{20}_D=1.5099.

Example 14



3 g of 4-hydroxymethyl-2-(3,4,4-trifluoro-3-butenylthio) oxazole and 1.5 g of triethylamine were dissolved in 50 ml of dichloromethane, to which a solution of 1 g of acetyl chloride in 20 ml of dichloromethane was added dropwise under ice cooling. After stirring at room temperature for 8 hours, the mixture was washed with water and dried with anhydrous sodium sulfate. After the solvent was distilled off under reduced pressure, the residue was treated by column chromatography to obtain 2.7 g of 4-acetoxymethyl-2-(3,4,4-trifluoro-3-butenylthio)oxazole (yield 76.5%). n^{20}_D=1.4752.

Example 15

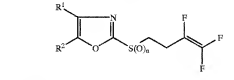


1.2 g of 4-chloromethyl-2-(3,4,4-trifluoro-3-butenylthio) oxazole, 1 g of potassium cyanide, 0.1 g of 18-crown-6-ether and 0.1 g of sodium iodide were added to 20 ml of dimethylformamide (dehydrated) and stirred at 100° C. for 20 hours. After cooling, 200 ml of water was added to the reaction mixture and extracted with 100 ml of diethyl ether twice. The extracted diethyl ether layers were put together, washed with 200 ml of water, dried with magnesium sulfate and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography (eluent: hexane:dichloromethane=2:1) to obtain 0.3 g of 4-cyanomethyl-2-(3,4,4-trifluoro-3-butenylthio)oxazole as colorless oil (yield 15.6%). n^{20}_D=1.4844.

The compounds of the formula (I) of the present invention, synthesized by the similar manner to the processes described in the above-mentioned Synthesis Examples 1–9 are shown in Table 1. And the compounds of Synthesis Examples 1–15 are also shown in Table 1.

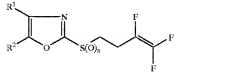
In Table 1, Me represents methyl, Et represents ethyl, n-Pr represents n-propyl, i-Pr represents isopropyl, n-Bu represents n-butyl, t-Bu represents t-butyl, n-Pen represents n-pentyl, n-Hexe represents n-hexyl and Ph represents phenyl.

TABLE 1



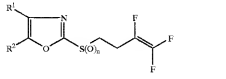
Compound No.	R^1	R^2	n	mp or n^{20}_D
1	H	H	0	1.4631
2	H	H	1	1.4820
3	H	H	2	1.4705
4	H	Cl	0	1.4830
5	H	Cl	1	1.4980
6	H	Cl	2	1.4805
7	H	Br	0	1.5029
8	H	Br	1	1.5110
9	H	Br	2	
10	H	I	0	
11	H	I	1	
12	H	I	2	
13	H	Me	0	
14	H	Me	1	
15	H	Et	0	
16	Me	H	0	1.4665
17	Me	H	1	1.4788
18	Me	H	2	1.4641
19	Me	Me	0	1.4702
20	Me	Me	1	1.4825
21	Me	Me	2	1.4683
22	Me	Et	0	
23	Me	n-Pr	0	
24	Et	H	0	
25	Et	H	1	
26	Et	H	2	
27	n-Pr	H	0	
28	n-Pr	H	1	
29	i-Pr	H	0	
30	n-Bu	H	0	
31	n-Bu	H	2	
32	t-Bu	H	0	
33	n-Pen	H	0	
34	$ClCH_2$	H	0	1.4933
35	$ClCH_2$	Cl	0	
36	$ClCH_2$	Cl	1	
37	$ClCH_2$	Cl	2	
38	$BrCH_2$	H	0	
39	$BrCH_2$	Br	0	1.4963
40	$BrCH_2$	Br	2	1.4745
41	$BrCH_2$	$BrCH_2$	0	
42	$BrCH_2$	$BrCH_2$	1	

TABLE 1-continued



Compound No.	R^1	R^2	n	mp or n^{20}_D
43	$BrCH_2$	$BrCH_2$	2	
44	$BrCH_2$	Me	0	
45	Me	$BrCH_2$	0	
46	H	$MeOCH_2$	0	1.4705
47	H	$MeOCH_2$	1	1.4812
48	H	$MeOCH_2$	2	
49	H	$EtOCH_2$	0	
50	H	$EtOCH_2$	2	
51	H	i-$PrOCH_2$	0	
52	Cl	H	0	1.4813
53	Cl	H	1	
54	Cl	H	2	1.4785
55	Cl	Me	0	
56	Cl	Me	1	
57	Cl	Me	2	
58	Cl	Et	0	
59	Cl	Et	1	
60	Cl	Et	2	
61	Cl	n-Pr	0	
62	Cl	i-Pr	0	
63	Cl	Cl	0	
64	Br	H	0	1.5012
65	Et	H	1	1.5226
66	Br	H	2	
67	I	H	0	
68	I	H	2	
69	Me	MeOOC	0	1.4972
70	Me	MeOOC	1	
71	Me	MeOOC	2	1.4710
72	Me	EtOOC	0	
73	Me	EtOOC	1	
74	Me	n-PrOOC	0	
75	$HOCH_2$	H	0	1.4910
76	$HOCH_2$	H	1	
77	$HOCH_2$	H	2	
78	$MeOCH_2$	H	0	1.4710
79	$MeOCH_2$	H	1	
80	$MeOCH_2$	H	2	1.4700
81	$EtOCH_2$	H	0	
82	$EtOCH_2$	H	1	
83	$EtOCH_2$	H	2	
84	n-$PrOCH_2$	H	0	
85	i-$PrOCH_2$	H	0	
86	$MeSCH_2$	H	0	1.5099
87	$MeSCH_2$	H	1	
88	$MeSCH_2$	H	2	
89	$EtSCH_2$	H	0	
90	$EtSCH_2$	H	1	
91	$EtSCH_2$	H	2	
92	n-$PrSCH_2$	H	0	
93	i-$PrSCH_2$	H	0	
94	$MeCOOCH_2$	H	0	1.4752
95	$MeCOOCH_2$	H	1	
96	$MeCOOCH_2$	H	2	1.4765
97	$EtCOOCH_2$	H	0	
98	$EtCOOCH_2$	H	1	
99	$EtCOOCH_2$	H	2	
100	n-$PrCOOCH_2$	H	0	
101	i-$PrCOOCH_2$	H	0	
102	CF_3COOCH_2	H	0	1.4430
103	CF_2COOCH_2	H	1	
104	CF_2COOCH_2	H	2	1.4388
105	$NCCH_2$	H	0	1.4844
106	$NCCH_2$	H	1	
107	$NCCH_2$	H	2	
108	Ph	H	0	1.5455
109	Ph	H	1	

TABLE 1-continued



Compound No.	R^1	R^2	n	mp or n^{20}_D
110	Ph	H	2	1.5368
111	2-F-Ph	H	0	
112	4-Cl-Ph	H	0	
113	4-Cl-Ph	H	2	
114	3-Me-Ph	H	0	
115	4-CF_3-Ph	H	0	
116	4-MeO-Ph	H	0	
117	4-MeS-Ph	H	0	
118	4-$MeSO_2$-Ph	H	2	
119	4-CF_3O-Ph	H	0	
120	4-CF_3S-Ph	H	0	
121	4-Ph-Ph	H	0	
122	4-PhO-Ph	H	0	
123	4-CN-Ph	H	0	
124	4-NO_2-Ph	H	0	
125	3,4-diMe-Ph	H	0	
126	2,4-diCl-Ph	H	0	
127	$MeSO_2O$	H	0	1.4830
128	$MeSO_2O$	H	1	
129	$MeSO_2O$	H	2	
130	$EtSO_2O$	H	0	
131	$EtSO_2O$	H	2	
132	n-Hex	H	0	
133	H	n-Pr	0	
134	H	iso-Pr	0	
135	H	iso-Pr	2	
136	H	n-Bu	0	
137	H	n-Pen	0	
138	H	n-Hex	0	

Preparation Examples (Starting Materials)

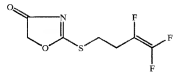
Example (A)

16.5 g of potassium thiocyanate were added to 400 ml of ethanol, to which 15 ml of concentrated hydrochloric acid was added little by little under cooling and stirred for 1 hour. An ethanol solution of thiocyanic acid was obtained by filtration of the reaction mixture. In another flask 300 ml of water and 19.0 g of dihydroxyfumaric acid were stirred at 60° C. for 1 hour to obtain an aqueous solution of glycolaldehyde through decarboxylation. The solutions were mixed and refluxed for 15 hours. After the reaction was finished, the solvent was distilled off under reduced pressure and the residue was purified by column chromatography (eluent: ethyl acetate:dichloromethane=1:9) to obtain 4 g of 2-mercaptooxazole as white crystals (yield 30.8%). m.p. 149–150° C.

Example (B)

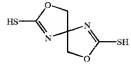
32 ml of 35% formalin, 46 ml of water and 165 ml of concentrated hydrochloric acid were added to a mixture of 65 g of potassium cyanide and 97 g of potassium thiocyanate, and stirred at room temperature for one night. After filtering the deposited crystals, the filtrate was refluxed for 2 hours and alkalized with an aqueous solution of sodium hydroxide. After washing with ether, the solution was acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The ethyl acetate layer was dried with anhydrous sodium sulfate and the solvent was distilled off under reduced pressure to obtain 26.1 g of 4-oxazolidinon-2-thione (yield 18%). {J. Med. Chem. 37(2), 322–8 (1994)}.

Example (C)



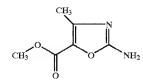
1 g of 4-oxazolidinon-2-thione and 1.8 g of triethylamine were dissolved in 43 ml of tetrahydrofuran, to which 1.9 g of 4-bromo-1,1,2-trifluoro-1-butene and 0.2 g of 4-dimethylaminopyridine were added and refluxed for 4 hours. After cooling and distilling off the solvent under reduced pressure, ether and water were added and stirred vigorously. After washing the ether layer with aqueous solution of sodium hydroxide and dried with anhydrous sodium sulfate, the solvent was distilled off under reduced pressure. The residue was treated by column chromatography (eluent: dichloromethane) to obtain 1 g of 2-(3,4,4-trifluoro-3-butenylthio)oxalin-4-one (yield 52%). n^{25}_{D}=1.4964.

Example (D)



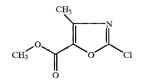
40 ml of concentrated hydrochloric acid was added little by little to a suspension of 35.0 g of potassium thiocyanate in 300 ml of ethanol, under ice cooling and stirred for 1 hour. By filtering the deposited crystals, an ethanol solution of thiocyanic acid was obtained. A solution of 15.0 g of dihydroxyacetone in 100 ml of ethanol was added to the above-mentioned ethanol solution of thiocyanic acid and refluxed for 15 hours. After finishing the reaction, the solvent was distilled off under reduced pressure and the residue was washed with 100 ml of dichloromethane and filtered. The filtered crystals were recrystallized from ethanol to obtain 28.4 g of 3,8-dioxa-1,6-diazaspiro[4,4]nona-1,6-diene-2,7-dithiol (yield 89.6%). mp. 200–202° C.

Example (E)



150 g of methyl 2-chloroacetoacetate and 180 g of urea were dissolved in 600 ml of methanol and refluxed for 36 hours. After cooling, the deposited crystals were filtered, suspended in 2N sodium hydroxide solution and extracted with ethyl acetate. After washing with water and drying with anhydrous sodium sulfate, the solvent was distilled off under reduced pressure. The residue was recrystallized from acetonitrile to obtain 10 g of 2-amino-5-methoxycarbonyl-4-methyloxazole. mp. 199–201° C. (point of decomposition).

Example (F)



To a suspension of 4.84 g of cupric chloride and 3.4 g of tert-butyl nitrite in 150 ml of acetonitrile, 4.7 g of 2-amino-5-methoxycarbonyl-4-methyloxazole was added at below 10° C. under argon stream and stirred at room temperature for 2 hours. After treatment with 2N hydrochloric acid, the mixture was extracted with ether, washed with water, dried with anhydrous sodium sulfate and the solvent was distilled off under reduced pressure. The residue was treated by silica gel column chromatography to obtain 2.1 g of 2-chloro-5-methoxycarbonyl-4-methyloxazole. mp. 69–71° C.

Use Examples

Example 1

Test Against Meloidogyne spp. (Soil Pot Test)

Preparation of Test Agent:

1 Part of the active compound is impregnated to 99 parts of pumice to make fine gramules.

Test Method:

A compound of the formula (I) was added to the soil contaminated by *Meloidogyne incognita* so that the chemical concentration would be 10 ppm and homogeneously mixed by stirring. A pot (1/5000 are) was filled with the soil. About 20 seeds of tomato (variety: Kurihara) were sown per pot. After cultivation in a greenhouse for 4 weeks, they were carefully pulled out not to damage the roots and the root knot index and the controlling effect were determined as follows.

Degree of damage

0: No knot was formed

1: A few knots were formed

2: Knots were formed to a medium extent

3: Knots were formed to an intense extent

4: Knots were formed to the most intense extent (corresponds to non-treatment).

$$\text{Root knot index} = \frac{\sum(\text{degree of damage} \times \text{number of individuals})}{\text{Total number of tested individuals} \times 4} \times 100$$

$$\text{Controlling effect} = \frac{\left(\begin{array}{c}\text{(Root knot index at non-treated area} - \\ \text{Root knot index at treated area)}\end{array}\right)}{\text{(Root knot index at non-treated area)}} \times 100$$

Evaluation of the ability of the tested compounds to control the nematodes was done according to the values obtained for the controlling effect and with the following standards.

a: Controlling effect 100–71%

b: Controlling effect 70–50%

c: Controlling effect less than 50%

d: Controlling effect 0%

In this test, the compounds of the Preparation Examples 1, 2, 3, 4, 5, 6, 8, 16, 17, 18, 19, 20, 21, 39, 46, 52, 54, 64, 65, 71, 75, 78, 80, 86, 96, 108, 110 and 127 showed controlling effect a.

Formulation Examples

Example a)

(Granule)

To a mixture of 10 parts of a compound of the present invention (e.g. Example. 1), 30 parts of bentonite (montmorillonite), 58 parts of talc and 2 parts of ligninsulfonate salt, 25 parts of water are added, well kneaded, made into granules of 10–40 mesh with the help of an extrusion granulator and dried at 40–50° C. to obtain granules.

Example b)

(Granule)

95 parts of clay mineral particles having particle diameter distribution of 0.2–2 mm are put into a rotary mixer. While rotating it, 5 parts of a compound according to the present invention (e.g Example 2) are sprayed together with a liquid diluent, wetted uniformly and dried at 40–50° C. to obtain granules.

Example c

(Emulsifiable Concentrate)

30 parts of a compound according to the present invention (e.g. Example 3), 55 parts of xylene, 8 parts of polyoxyethylene alkyl phenyl ether and 7 parts of calcium alkylbenzenesulfonate are mixed and stirred to obtain an emulsifiable concentrate.

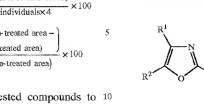
Example d)

(Wettable Powder)

15 parts of a compound according to the present invention (e.g. Example 1), 80 parts of a mixture of white carbon (hydrous amorphous silicon oxide fine powders) and powder clay (1:5), 2 parts of sodium alkylbenzenesulfonate and 3 parts of sodium alkylnaphthalenesulfonate-formalin-condensate are crushed and mixed to obtain a wettable powder.

What is claimed is:

1. A compound of the formula (I)



(I)

wherein

R^1 represents hydrogen; halogen; alkyl that is unsubstituted or substituted with halogen, hydroxy, alkoxy, alkylthio, alkylcarbonyloxy, haloalkylcarbonyloxy, or cyano; or alkylsulfonyloxy;

R^2 represents hydrogen; halogen; alkyl that is unsubstituted or substituted with alkoxy or halogen; or alkoxycarbonyl; and

n represents 0, 1, or 2,

with the proviso that if R^1 represents alkyl, then R^2 does not represent halogen.

2. A componnd of the formula (I) according to claim **1** wherein

R^1 represents hydrogen; halogen; C_{1-6} alkyl that is unsubstituted or substituted with halogen hydroxy, C_{1-3} alkoxy, C_{1-3} alkylthio, C_{1-3} alkylcarbonyloxy, trifluoromethylcarbonyloxy or cyano; or C_{1-4} alkylsulfonyloxy;

R^2 represents hydrogen; halogen; C_{1-6} alkyl that is unsubstituted or substituted with C_{1-3} alkoxy or halogen; or C_{1-4} alkoxycarbonyl; and

n represents 0 or 2.

3. A compound of the formula (I) according to claim **1** wherein

R^1 represents hydrogen; fluoro, chloro, or bromo; C_{1-6} alkyl that is unsubstituted or substituted with fluoro, chloro, bromo, hydroxy, C_{1-3} alkoxy, C_{1-3} alkylthio, C_{1-3} alkylcarbonyloxy, trifluoromethylcarbonyloxy or cyano; or methylsulfonyloxy or ethylsulfonyloxy;

R^2 represents hydrogen; fluoro, chloro, or bromo; C_{1-4} alkyl that is unsubstituted or substituted with C_{1-3} alkoxy or bromo; or C_{1-3} alkoxycarbonyl; and

n represents 0.

4. A compound of the formula (I) according to claim **1** wherein

R^1 represents hydrogen; halogen; methyl, ethyl, n- or i-propyl, n-, i-, s-, or t-butyl, n-pentyl, or n-hexyl, each of which is optionally substituted with halogen, hydroxy, methoxy, ethoxy, n- or i-propoxy, methylthio, ethylthio, n- or i-propylthio, methylcarbonyloxy, ethylcarbonyloxy, n- or i-propylcarbonyloxy, trifluoromethylcarbonyloxy, or cyano; methylsulfonyloxy, ethylsulfonyloxy, n- or i-propylsulfonyloxy, or n-, i-, s-, or t-butylsulfonyloxy;

R^2 represents hydrogen; halogen; methyl, ethyl, n-or i-propyl, n-, i-, s-, or t-butyl, n-pentyl, or n-hexyl, each of which is optionally substituted with methoxy, ethoxy, n- or i-propoxy, or halogen; or methoxycarbonyl, ethoxycarbonyl, n- or -propoxycarbonyl or n-, i-, s-, or t-butoxycarbonyl; and

n represents 0, 1 or 2.

5. A compound of the formula (I) according to claim **1** wherein

R^1 represents hydrogen; fluoro, chloro, or bromo; methyl, ethyl, n- or i-propyl, or n-, i-, s-, or t-butyl, each of

which is optionally substituted with fluoro, chloro, bromo, hydroxy, methoxy, ethoxy, n- or i-propoxy, methylthio, ethylthio, n- or i-propylthio, trifluoromethylcarbonyloxy, or cyano; or methylsulfonyloxy or ethylsulfonyloxy;

R^2 represents hydrogen; fluoro, chloro, or bromo; methyl, ethyl, n- or i-propyl, or n-, i-, s-, or t-butyl, each of which is optionally substituted with methoxy, ethoxy, n- or i-propoxy, or bromo; or methoxycarbonyl, ethoxycarbonyl, or n- or i-propoxycarbonyl; and

n represents 0, 1 or 2.

6. A process for preparing a compound of formula (I) according to claim **1** comprising

(a) for compounds of formula (I) in which

R^1 represents hydrogen; or alkyl that is optionally substituted with halogen, hydroxy, alkoxy, alkylthio, alkylcarbonyloxy, haloalkyl-carbonyloxy, or cyano;

R^2 represents hydrogen or alkyl that is optionally substituted with alkoxy or halogen; and

n represents **0**,

reacting a compound of the formula (II)

(II)

wherein

R^{1a} represents hydrogen; or alkyl that is optionally substituted with halogen, hydroxy, alkoxy, alkylthio, alkylcarbonyloxy, haloalkyl-carbonyloxy, or cyano;

R^{2a} represents hydrogen or alkyl that is optionally substituted with alkoxy or halogen; and

n represents **0**,

with 4-bromo-1,1,2-trifluoro-1-butene in the presence of an inert solvent and optionally in the presence of an acid binder; or

(b) for compounds of formula (I) in which

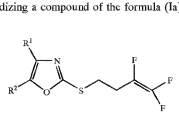
R^1 represents hydrogen; or alkyl that is optionally substituted with halogen, hydroxy, alkoxy, alkylthio, alkylcarbonyloxy, haloalkyl-carbonyloxy, or cyano;

R^2 represents hydrogen or alkyl that is optionally substituted with alkoxy or halogen; and

n represents 1 or 2,

oxidizing a compound of the formula (Ia)

(Ia)



wherein

R^1 represents hydrogen; or alkyl that is optionally substituted with halogen, hydroxy, alkoxy, alkylthio, alkylcarbonyloxy, haloalkylcarbonyloxy, or cyano; and

R^2 represents hydrogen or alkyl that is optionally substituted with alkoxy or halogen,

in the presence of an inert solvent; or

(c) for compounds of formula (I) in which

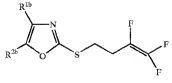
R^1 represents hydrogen or haloalkyl;

R^2 represents halogen; and

n represents 0,

reacting a compound of the formula (Ib)

(Ib)



wherein

R^{1b} represents hydrogen or alkyl; and

R^{2b} represents hydrogen,

with a halogenating agent in the presence of an inert solvent; or

(d) for compounds of formula (I) in which

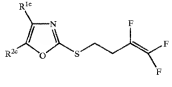
R^1 represents haloalkyl;

R^2 represents hydrogen or haloalkyl; and

n represents 0,

reacting a compound of the formula (Ic)

(Ic)



wherein

R^{1c} represents alkyl; and

R^{2c} represents hydrogen or alkyl,

with a halogenating agent in the presence of an inert solvent; or

(e) for compounds of formula (I) in which

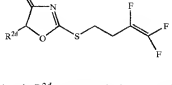
R^1 represents halogen;

R^2 represents hydrogen or alkyl; and

n represents 0,

reacting a compound of the formula (III)

(III)



wherein R^{2d} represents hydrogen or alkyl,

with a halogenating agent in the presence of an inert solvent and optionally in the presence of an organic base; or

(f) for compounds of the formula (I) in which

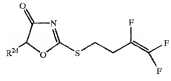
R^1 represents alkylsulfonyloxy;

R^2 represents hydrogen or alkyl; and

n represents 0,

reacting a compound of formula (III)

(III)



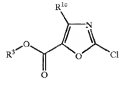
wherein R^{2d} represents hydrogen or alkyl, with a compound of the formula

$$R^{1d}SO_2Cl \quad (IV)$$

wherein R^{2d} represents alkyl,
in the presence of an inert solvent and optionally in the presence of an inorganic or organic base; or

(g) for compounds of formula (I) in which
R^1 represents alkyl;
R^2 represents alkoxycarbonyl; and
n represents 0,
reacting a compound of the formula (IV)

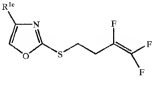
(V)



wherein
R^{1c} represents alkyl; and
R^3 represents alkyl,
with thiourea to form a product that is then reacted with 4-bromo-1,1,2-trifluoro-1-butene in the presence of an inert solvent; or

(h) for compounds of formula (I) in which
R^1 represents hydrogen;
R^2 represents alkoxyalkyl; and
n represents 0,
reacting a compound of the formula (Id)

(Id)



wherein R^{1e} represents hydrogen,
with a compound of the formula (VI)

$$R^4—Br \quad (VI)$$

wherein R^4 represents alkoxyalkyl, in the presence of an inert solvent.

7. A nematicidal composition comprising at least one trifluorobutene of the formula (I) of claim **1** and one or more extenders.

8. A process for combating nematodes comprising allowing an effective amount of a formulation or application form comprising 0.000001 to 100% by weight of a trifluorobutene of the formula (I) of claim **1** act on nematodes and/or the habitat of nematodes.

* * * * *

Exhibit E

US006683107B2

(12) **United States Patent**
Dollings et al.

(10) **Patent No.: US 6,683,107 B2**
(45) **Date of Patent: Jan. 27, 2004**

(54) **FURANS, BENZOFURANS, AND THIOPHENES USEFUL IN THE TREATMENT OF INSULIN RESISTANCE AND HYPERGLYCEMIA**

(75) Inventors: **Paul J. Dollings**, Newtown, PA (US); **Robert E. McDevitt**, Somerset, NJ (US); **Folake O. Adebayo**, Cranbury, NJ (US)

(73) Assignee: **Wyeth**, Madison, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/215,311**

(22) Filed: **Aug. 8, 2002**

(65) **Prior Publication Data**

US 2003/0073709 A1 Apr. 17, 2003

Related U.S. Application Data

(62) Division of application No. 09/887,672, filed on Apr. 23, 2001, now Pat. No. 6,451,845, which is a continuation of application No. 09/564,469, filed on May 4, 2000, now Pat. No. 6,248,764, which is a division of application No. 09/307,691, filed on May 10, 2000, now Pat. No. 6,103,708.

(60) Provisional application No. 60/126,416, filed on May 12, 1998.

(51) **Int. Cl.**[7] **A61K 31/381**; A61K 31/341; C07D 333/02; C07D 323/02

(52) **U.S. Cl.** **514/438**; 514/461; 514/465; 549/29; 549/429

(58) **Field of Search** 514/438, 336, 514/449, 361, 363, 365, 403, 406, 156, 461, 465, 448; 549/29, 429

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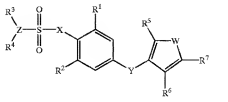
Primary Examiner—Rita Desai
(74) *Attorney, Agent, or Firm*—Michael R. Nagy

(57) **ABSTRACT**

This invention provides compounds of Formula I having the structure



(I)

wherein

R^1 and R^2 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, halogen, perfluoroalkyl of 1–6 carbon atoms, cycloalkyl of 3–8 carbon atoms, thienyl, furyl, phenyl or phenyl substituted with trifluoromethyl, chloro, methoxy, or trifluoromethoxy;

R^3 and R^4 are each, independently, hydrogen, carboxyl, hydroxyl, hydoxyalkyl of 1–6 carbon atoms, alkoxy of 1–6 carbon atoms, perfluoroalkoxy of 1–6 carbon atoms, alkanoyloxy of 2–7 carbon atoms, perfluoroalkanoyloxy of 2–7 carbon atoms, arylalkoxy of 7–15 carbon atoms, aryloxy of 6–12 carbon atoms, aroyloxy of 6–12 carbon atoms, aryloxycarbonyl of 7–13 carbon atoms, alkoxycarbonyl of 2–7 carbon atoms, perfluoroalkoxycarbonyl of 2–7 carbon atoms, alkyl of 1–6 carbon atoms, perfluoroalkyl of 1–6 carbon atoms, alkylamino of 1–6 carbon atoms, dialkylamino of 1–6 carbon atoms per alkyl group, tetrazolyl, mercapto, nitrile, nitro, amino, $—NHSO_2CF_3$, carbamoyl, carboxyaldehyde, halogen, acylamino, 3-hydroxy-cyclobut-3-ene-4-yl-1,2-dione, pyridyl, isoxazolyl, pyrimidyl or pyrimidyl substituted with mercapto, or tetronic acid;

R^5 is hydrogen, alkyl of 1–6 carbon atoms, perfluoroalkyl of 1–6 carbon atoms, naphthalenylmethyl, benzyl or benzyl substituted with halogen,

R^6 and R^7 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, or perfluoroalkyl of 1–6 carbon atoms, or R^6 and R^7 may be taken together as a diene unit having the structure $—CH═CH—CH═CH—$;

W is S or O,

X is $—NR^8CH_2—$, $—NR^9—$, or O;

R^8 is hydrogen or alkyl;

Y is carbonyl, methylene, ethyl, or $—NHCH_2—$;

Z is phenyl, pyridyl, naphthyl, thienyl, furyl, pyrrolyl, pyrazolyl, isoxazolyl, or isothiazolyl;

or a pharmaceutically acceptable salt thereof, which are useful in treating metabolic disorders related to insulin resistance or hyperglycemia.

3 Claims, No Drawings

FURANS, BENZOFURANS, AND THIOPHENES USEFUL IN THE TREATMENT OF INSULIN RESISTANCE AND HYPERGLYCEMIA

BACKGROUND OF THE INVENTION

The prevalence of insulin resistance in glucose intolerant subjects has long been recognized. Reaven et al (*American Journal of Medicine* 1976, 60, 80) used a continuous infusion of glucose and insulin (insulin/glucose clamp technique) and oral glucose tolerance tests to demonstrate that insulin resistance existed in a diverse group of nonobese, nonketotic subjects. These subjects ranged from borderline glucose tolerant to overt, fasting hyperglycemiaa. The diabetic groups in these studies included both insulin dependent (IDDM) and noninsulin dependent (NIDDM) subjects.

Coincident with sustained insulin resistance is the more easily determined hyperinsulinemia, which can be measured by accurate determination of circulating plasma insulin concentration in the plasma of subjects. Hyperinsulinemia can be present as a result of insulin resistance, such as is in obese and/or diabetic (NIDDM) subjects and/or glucose intolerant subjects, or in IDDM subjects, as a consequence of over injection of insulin compared with normal physiological release of the hormone by the endocrine pancreas.

The association of hyperinsulinemia with obesity and with ischernic diseases of the large blood vessels (e.g. atherosclerosis) has been well established by numerous experimental, clinical and epidemiological studies (summarized by Stout, *Metabolism* 1985, 34, 7, and in more detail by Pyorala et al, *Diabetes/Metabolism Reviews* 1987, 3, 463). Statistically significant plasma insulin elevations at 1 and 2 hours after oral glucose load correlates with an increased risk of coronary heart disease.

Since most of these studies actually excluded diabetic subjects, data relating the risk of atherosclerotic diseases to the diabetic condition are not as numerous, but point in the same direction as for nondiabetic subjects (Pyorala et al). However, the incidence of atherosclerotic diseases in morbidity and mortality statistics in the diabetic population exceeds that of the nondiabetic population (Pyorala et al; Jarrett *Diabetes/Metabolism Reviews* 1989,5, 547; Harris et al, Mortality from diabetes, in Diabetes in America 1985).

The independent risk factors obesity and hypertension for atherosclerotic diseases are also associated with insulin resistance. Using a combination of insulin/glucose clamps, tracer glucose infusion and indirect calorimetry, it has been demonstrated that the insulin resistance of essential hypertension is located in peripheral tissues (principally muscle) and correlates directly with the severity of hypertension (DeFronzo and Ferrannini, *Diabetes Care* 1991, 14, 173). In hypertension of the obese, insulin resistance generates hyperinsulinemia, which is recruited as a mechanism to limit further weight gain via thermogenesis, but insulin also increases renal sodium reabsorption and stimulates the sympathetic nervous system in kidneys, heart, and vasculature, creating hypertension.

It is now appreciated that insulin resistance is usually the result of a defect in the insulin receptor signaling system, at a site post binding of insulin to the receptor. Accumulated scientific evidence demonstrating insulin resistance in the major tissues which respond to insulin (muscle, liver, adipose), strongly suggests that a defect in insulin signal transduction resides at an early step in this cascade, specifically at the insulin receptor kinase activity, which appears to be diminished (reviewed by Haring, *Diabetalogia* 1991, 34, 848).

Protein-tyrosine phosphatases (PTPases) play an important role in the regulation of phospboryiation of proteins. The interaction of insulin with its receptor leads to phosphorylation of certain tyrosine molecules within the receptor protein, thus activating the receptor kinase. PTPases dephosphorylate the activated insulin receptor, attenuating the tyrosine kinase activity. PTPases can also modulate post-receptor signaling by catalyzing the dephosphorylation of cellular substrates of the insulin receptor kinase. The enzymes that appear most likely to closely associate with the insulin receptor and therefore, most likely to regulate the insulin receptor kinase activity, include PTP1B, LAR, PTPα and SH-PTP2 (B. J. Goldstein, *J. Cellular Biochemistry* 1992, 48, 33; B. J. Goldstein, Receptor 1993, 3, 1–15,; F. Ahmad and B. J. Goldstein *Biochim. Biophys Acta* 1995, 1248, 57–69).

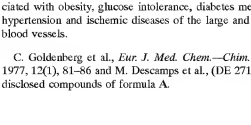
McGuire et al. (*Diabetes* 1991, 40, 939), demonstrated that nondiabetic glucose intolerant subjects possessed significantly elevated levels of PTPase activity in muscle tissue vs. normal subjects, and that insulin infusion failed to suppress PTPase activity as it did in insulin sensitive subjects.

Meyerovitch et al (*J. Clinical Invest.* 1989, 84, 976) observed significantly increased PTPase activity in the livers of two rodent models of IDDM, the genetically diabetic BB rat, and the STZ-induced diabetic rat. Sredy et al (*Metabolism*, 44, 1074, 1995) observed similar increased PTPase activity in the livers of obese, diabetic ob/ob mice, a genetic rodent model of NIDDM.

The compounds of this invention have been shown to inhibit PTPases derived from rat liver microsomes and human-derived recombinant PTPase-1B (hPTP-1B) in vitro. They are useful in the treatment of insulin resistance associated with obesity, glucose intolerance, diabetes mellitus, hypertension and ischemic diseases of the large and small blood vessels.

C. Goldenberg et al., *Eur. J. Med. Chem.—Chim. Ther.* 1977, 12(1), 81–86 and M. Descamps et al., (DE 2710047) disclosed compounds of formula A.

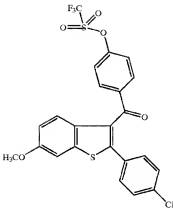
A



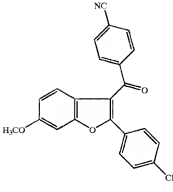
R^1 is H, Cl, NO_2, CH_3, OCH_3; R^2 is alkyl; R^3 is H, alkylaminomethyl

G. J. Cuilinan and K. J. Fahey (U.S. Pat. No. 5,596,106 A and WO 960201) disclose arylbenzo[b]thiophene and benzo[b]furan compounds B and C as cannabinoid receptor antagonists.

B

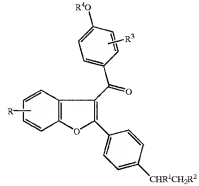


C



H. Grote (DE 3342624 A1) disclose Benzarone derivatives D for treating venous and arterial ailments.

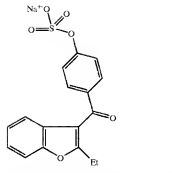
D



(R, R^1, R^2 and R^3 is H, alkoxy, acyloxy, OH, SO_3H; R^4 is H, acyl, HSO_2)

T. Eckert (DE 3110460 and *Arch. Pharm.* (Weinheim, Ger.) 1982, 315(6), 569–570 discloses sodium benzaron sulfate E.

E

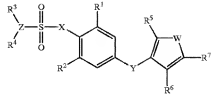


None of the above disclosures (A–E) contained the appropriate substitution necessary for in vitro PTPase inhibition activity.

DESCRIPTION OF THE INVENTION

This invention provides a compound of formula I having the structure

(I)



wherein

R^1 and R^2 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, halogen, perfluoroalkyl of 1–6 carbon atoms, cycloalkyl of 3–8 carbon atoms, thienyl, furyl, phenyl or phenyl substituted with trifluoromethyl, chloro, methoxy, or trifluoromethoxy;

R^3 and R^4 are each, independently, hydrogen, carboxy, hydroxy, hydoxyalkyl of 1–6 carbon atoms, alkoxy of 1–6 carbon atoms, perfluoroalkoxy of 1–6 carbon atoms, alkanoyloxy of 2–7 carbon atoms, perfluoroalkanoyloxy of 2–7 carbon atoms, arylalkoxy of 7–15 carbon atoms, aryloxy of 6–12 carbon atoms, aroyloxy of 7–13 carbon atoms, aryloxycarbonyl of 7–13 carbon atoms, alkoxycarbonyl of 2–7 carbon atoms, perfluoroalkoxycarbonyl of 2–7 carbon atoms, alkyl of 1–6 carbon atoms, perfluoroalkyl of 1–6 carbon atoms, alkylamino of 1–6 carbon atoms, dialkylamino of 1–6 carbon atoms per alkyl group, tetrazolyl, mercapto, nitrile, nitro, amino, —$NHSO_2CF_3$, carbamoyl, formyl, halogen, acylamino, 3-hydroxy-cyclobut-3-ene-4-yl-1,2-dione, or tetronic acid;

R^5 is hydrogen, alkyl of 1–6 carbon atoms, perfluoroalkyl of 1–6 carbon atoms, naphthalenylmethyl, benzyl or benzyl substituted with halogen,

R^6 and R^7 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, or perfluoroaLkyl of 1–6 carbon atoms, or R^6 and R^7 may be taken together as a diene unit having the structure —CH═CH—CH═CH—;

W is S or O,

X is —NR^9CH_2—, —NR^8—, or O;

R^8 is hydrogen or alkyl of 1–6 carbon atoms;

Y is carbonyl, methylene, —CH_2CH_2—, or —$NHCH_2$—;

Z is phenyl, pyridyl, naphthyl, thienyl, furyl, pyrrolyl, pyrazolyl, isoxazolyl, or isothiazolyl;

or a pharmaceutically acceptable salt thereof, which are useful in treating metabolic disorders related to insulin resistance or hyperglycemia.

Pharmaceutically acceptable salts can be formed from organic and inorganic acids, for example, acetic, propionic, lactic, citric, tartaric, succinic, fumaric, maleic, malonic, mandelic, malic, phthalic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, napthalenesulfonic, benzenesulfonic, toluenesulfonic, camphorsulfonic, and similarly known acceptable acids when a compound of this invention contains a basic moiety. Salts may also be formed from organic and inorganic bases, preferably alkali metal salts, for example, sodium, lithium, or potassium, when a compound of this invention contains a carboxylate or phenolic moiety, or similar moiety capable of forming base addition salts.

Alkyl includes both straight chain as well as branched moieties. Halogen means bromine, chlorine, fluorine, and iodine. It is preferred that the aryl portion of the aryl, arylkyl, arylalkoxy, aryloxy, aroyloxy, or aryloxycarbonyl substituent is a phenyl, naphthyl or 1,4-benzodioxan-5-yl group, with phenyl being most preferred. The aryl moiety may be optionally mono-, di-, or tri-substituted with a substituent selected from the group consisting of alkyl of 1–6 carbon atoms, alkoxy of 1–6 carbon atoms, trifluoromethyl, halogen, alkoxycarbonyl of 2–7 carbon atoms, alkylamino of 1–6 carbon atoms, and dialkylamino in which each of the alkyl groups is of 1–6 carbon atoms, nitro, cyano, $—CO_2H$, alkanoyloxy of 2–7 carbon atoms, and alkylcarbonyl of 2–7 carbon atoms.

The compounds of this invention may contain an asymmetric carbon atom and some of the compounds of this invention may contain one or more asymmetric centers and may thus give rise to optical isomers and diastereomers. While shown without respect to stereochemistry in Formula I, the present invention includes such optical isomers and diastereomers, as well as the racemic and resolved, enantiomerically pure R and S stereoisomers, as well as other mixtures of the R and S stereoisomers and pharmaceutically acceptable salts thereof.

Preferred compounds of this invention are those compounds of Formula I,

wherein

R^1 and R^2 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, halogen, perfluoroalkyl of 1–6 carbon atoms, cycloalkyl of 3–8 carbon atoms, thienyl, furyl, phenyl or phenyl substituted with trifluoromethyl, chioro, methoxy, or trifluoromethoxy;

R^3 and R^4 are each, independently, hydrogen, carboxy, hydroxy, alkoxy of 1–6 carbon atoms, perfluoroalkoxy of 1–6 carbon atoms, alkanoyloxy of 2–7 carbon atoms, perfluoroalkanoyloxy of 2–7 carbon atoms, aroyloxy of 7–13 carbon atoms, alkoxycarbonyl of 2–7 carbon atoms, aryloxycarbonyl of 7–13 carbon atoms, alkyl of 1–6 carbon atoms, perfluoroalkyl of 1–6 carbon atoms, tetrazolyl, mercapto, nitrile, amino, $—NHSO_2CF_3$, carbamoyl, formyl, acylamino of 2–7 carbon atoms;

R^5 is hydrogen, alkyl of 1–6 carbon atoms, naphthalenylmethyl, benzyl or benzyl substituted with halogen;

R^6 and R^7 are each, independently hydrogen or alkyl of 1–6 carbon atoms or R^6 and R^7 may be taken together as a diene unit having the structure $—CH=CH—CH=CH—$;

W is S or O,

X is $—NHCH_2—$, or O;

Y is carbonyl, methylene, $—CH_2CH_2—$, or $—NHCH_2—$;

Z is phenyl, pyridyl, naphthyl, thienyl, furyl, pyrrolyl, pyrazolyl, isoxazolyl, or isothiazolyl;

or a pharmaceutically acceptable salt thereof.

More preferred compounds of this invention are those compounds of Formula I,

wherein

R^1 and R^2 are each, independently, hydrogen, iodo, phenyl, thienyl, alkyl of 1–6 carbon atoms, bromo, or cycloalkyl of 3–8 carbon atoms,

R^3 and R^4 are each, independently, hydrogen, carboxy, hydroxy, methyl, or acetoxy;

R^5 is hydrogen, alkyl of 1–6 carbon atoms, naphthalenylmethyl, benzyl or benzyl substituted with bromine;

R^6 and R^7 are each, indepedently, hydrogen or methyl, or R^6 and R^7 may be taken together as a diene unit having the structure $—CH=CH—CH=CH—$;

Wis S or O,

X is $—NHCH_2—$, or O;

Y is carbonyl, methylene, $—CH_2CH_2—$. $—NHCH_2—$;

Z is phenyl, or pyrazolyl;

or a pharmaceutically acceptable salt thereof.

Specifically preferred compounds of the present invention are set forth below:

4-[2,6-Dibromo-4-(2-ethyl-benzofuran-3-carbonyl)-phenoxysulfonyl]-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[5'-(2-Butyl-benzofuran-3-carbonyl)-[1,1'; 3'1"]terphenyl-2'-yloxysulfonyl]-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[4-(2-Ethyl-benzofuran-3-carbonyl)-2,6-dimethyl-phenoxysulfonyl]-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[4-(2-Ethyl-benzofuran-3-carbonyl)-2,6-diisopropyl-phenoxysulfonyl]-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[4-(2-Benzyl4,5-dimethyl-furan-3-carbonyl)-2,6-diisopropyl-phenoxysulfonyl]-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[5'-(2-Ethyl-benzofuran-3-carbonyl)-[1,1'; 3',1"] terphenyl-2'-yloxysulfonyl]-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[5-(2-Benzyl-4,5-dimethyl-furan-3-carbonyl)-3-methyl-biphenyl-2-yloxysulfonyl]-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[4-(4,5-Dimethyl-2-naphthalen-2-ylmethyl-ftiran-3-carbonyl)-2,6-diisopropyl-phenoxysulfonyl]-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[4-[2-(2-Bromo-benzyl)-4,5-dimethyl-furan-3-carbonyl]-2,6-diisopropyl-phenoxysulfonyl}-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[4-[2-(3-Bromo-benzyl)-4,5-dimethyl-furan-3-carbonyl]-2,6-diisopropyl-phenoxysulfonyl}-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[4-[2-(4-Bromo-benzyl)4,5-dimethyl-furan-3-carbonyl]-2,6-diisopropyl-phenoxysulfonyl}-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

2-Acetoxy-4-[4-[2-(2-Bromo-benzyl)-4,5-dimethyl-furan-3-carbonyl]-2-cyclopentyl-phenoxysulfonyl]-benzoic acid or a pharmaceutically acceptable salt thereof.

2-Acetoxy-4-[4-[2-(4-Bromo-benzyl)-4,5-dimethyl-thiophene-3-carbonyl]-2-cyclopentyl-phenoxysulfonyl}-benzoic acid or a pharmaceutically acceptable salt thereof.

2-Acetoxy4-[4-(2-benzyl-4,5-dimethyl-tbiophene-3-carbonyl)-2-cyclopentyl-phenoxysulfonyl]-benzoic acid or a pharmaceutically acceptable salt thereof.

2-Acetoxy-4-[4-(2-benzyl4,5-dimethyl-furan-3-carbonyl)-2-cyclopentyl-phenoxysulfonyl]-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[4-(2-Benzyl-4,5-dimethyl-furan-3-carbonyl)-2,6-diethyl-phenoxysulfonyll-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

1-Methyl-1H-pyrazole-4-sulfonic acid 4-[2-(4-bromo-benzyl)-4,5-dimethyl-thiophene-3-carbonyl]-2-cyclopentyl-phenyl ester or a pharmaceutically acceptable salt thereof.

4-[4-[2-(2-Bromo-benzyl)-4,5-dimethyl-furan-3-carbonyl]-2-cyclopentyl-phenoxysulfonyl}-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-{4-[2-(4-Bromo-benzyl)-4,5-dirnethyl-thiophene-3-carbonyl]-2-cyclopentyl-phenoxysulfonyl}-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[4-(2-Benzyl-4,5-dimethyl-thiophene-3-carbonyl)-2-cyclopentyl-phenoxysulfonyl]-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[4-(2-Benzyl-4,5-dimethyl-furan-3-carbonyl)-2-cyclopentyl-phenoxysulfonyl]-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-{4-[2-(4-Bromo-benzyl)-4,5-dimethyl-furan-3-carbonyl]-2-cyclopentyl-phenoxysulfonyl}-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-[4-(2-Butyl-benzofuran-3-ylmethyl)-phenoxysulfonyl]-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof

4-[4-(2-Butyl-benzofuran-3-carbonyl)-phenoxysulfonyl]-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-{4-[2-(2-Butyl-benzofuran-3-yl)-ethyl]-phenoxysulfonyl}-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

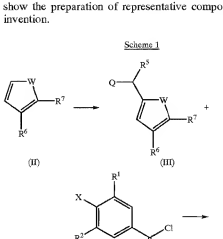
2-Acetoxy4-{4-[2-(2-butyl-benzofuran-3-yl)-ethyl]-phenoxysulfonyl}-benzoic acid or a pharmaceutically acceptable salt thereof.

4-{4-[(2-Butyl-benzofuran-3-ylmethyl)-arnino]-phenoxysulfonyl}-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

4-{4-[2-(2-Benzyl-benzo[b]thiophen-3-yl)thyl]-phenoxysulfonyl}-2-hydroxy-benzoic acid or a pharmaceutically acceptable salt thereof.

The compounds of this invention were be prepared according to the following schemes from commercially available starting materials or starting materials which can be prepared using to literature procedures. These schemes show the preparation of representative compounds of this invention.

Scheme 1



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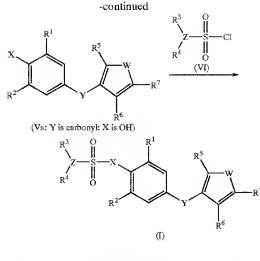


In Scheme 1, 2, 3-dimethylthiophene (II: W is S) is prepared from commercially available 3-methyl-thiophenecarboxaldehyde using Wolff-Kishner conditions (hydrazine followed by KOHiethylene glycol reflux). Compound (U) is treated with one to 1.3 molar equivalents of an alkyl lithium reagent such as N-butyl lithium most preferably in a non-protic solvent such as THF at temperatures ranging from −78° C. to room temperature under an inert atmosphere such as nitrogen or argon to provide the 2-lithiated-thiophene or furan derivative. This lithiated analog is reacted in situ with one or more molar equivalents of benzaldehyde, generally at −78° C. to room temperature for 5 min to 3 h to provide the compound of formula (III: Q is OH). The hydroxy group of (III: Q is OH) can be removed by a number of reduction procedures such as hydrogenation using palladium catalysis to produce the compound of formula (III: Q is H) but is most conveniently removed using the method of Nataitis, et. al. (*Org. Prep. and Proced. Int.* 1991, 23, 403–411) in which (III: Q is OH; W is S or O) is stirred with one to ten molar equivalents of sodium borohydride in a suitable solvent such as ether, THF or dichioromethane at 0° C. to room temperature and one to fifty molar equivalents of trifluoroacetic acid is slowly added over a 15 min to 3 h period to produce the compound of formula (III: Q is H). Alternatively, the 2-lithiated analog of compound (II) in a nonprotic solvent such as THF can be reacted with one or more molar equivalents of a benzyl halide such as benzyl bromide ($PhCH_2Br$) at −78° C. to room temperature to directly provide the compound of formula (III: Q is H, W is S or O).

The compounds of formula (III: Q is H) can be acylated with one or more molar equivalents of a commercially available benzoic acid chloride of formula (IV: X is O-alkyl) to produce the acylated derivative of formula (V: X is O-alkyl). This acylation is accomplished most readily using a one to five molar equivalents of a Lewis acid catalyst such as tin tetrachloride or aluminum chloride in an inert solvent such as dichioromethane, 1,2-dichloroethane or carbon disulfide, generally at temperatures such as −78° C. to room temperature. The benzoic acid chloride (IV: X is —O-alkyl), is prepared from the corresponding benzoic acid by standard procedures using reagents such as oxalyl chloride and thionyl chloride. The starting benzoic acid of the benzoic acid chloride (IV: X is O-alkyl) is commercially available or can be easily prepared by known procedures For example, the acid starting material for benzoic acid chloride (IV) can be prepared using a modification of the method of Schuster, et al., *J. Org. Chem.* 1988, 53, 5819. Thus commercially

available 2, 6-diisopropyl phenol is brominated in the 4-position (bromine/acetic acid), methylated (iodomethane/potassium carbonate/DMF), reacted with n-butyl lithium to effect lithium halogen exchange and the resultant organolithium species is reacted with carbon dioxide to provide 3, 5-diisopropyl, 4-methoxy benzoic acid. Alternatively, the commercially available 2,6-(mono or disubstituted)phenols can be methylated (iodomethane/potassium carbonate/DMF), acylated in the 4-position with 2-chlorobenzoyl chloride in the presence of aluminum chloride in an inert solvent such as dichloromethane, generally at ambient temperature and reacted with potassium-t-butoxide in H_2O/ethylene glycol dimethyl ether at ambient temperature to give the desired 2,6-(mono or disubstituted)benzoic acid.

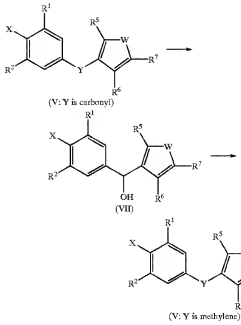
The conversion of the alkyl ether compound (V: X is O-alkyl) to the phenol compound (Va: X is OH) is generally best accomplished using one to ten molar equivalents of a strong Lewis acid such as a trihaloborane, most conveniently tribromoborane. The reaction is best performed at −78° C. with warming to 0° C.

The compounds of formula (Va: X is OH) can be sulfonylated on the phenolic oxygen using one or more molar equivalents of suitable sulfonylating agent (VI) to provide the sulfonic acid esters of formula (I: Y is carbonyl). The sulfonylating agent (VI) is generally a aryl or heteroaryl sulfonic acid chloride. The reaction is run under standard conditions using a suitable base such sodium hydride, pyridine or Tris base in an appropriate solvent such as dichloromethane, THF or H_2O at temperatures from 0° C. to ambient temperature. The starting aryl or heteroaryl sulfonic acid chloride is commercially available or can be easily prepared by known procedures. The aryl or heteroaryl sulfonic acid chloride can be prepared by reacting the aryl or heteroaryl sulfonic acid with one or more molar equivalents of oxalyl chloride or thionyl chloride, in a suitable solvent such as dichloromethane, chloroform or diethyl ether, to afford the aryl or heteroaryl sulfonic acid chloride. This reaction is often catalyzed by adding small amounts (0.01 to 0.1 molar equivalents) of dimethylformamide. Alternatively, the aryl or heteroaryl sulfonic acid chloride can prepared using a modification of Barraclough, et al., *Arch. Pharm.* (Weinheim) 1990, 323, 507. Thus, the aniline of commercially available 4-aminosalicylic acid sodium salt dihydrate is diazotized with sodium nitrite in HOAc/HCl at −10° C. and the subsequent the diazonium salt can converted to the sulfonyl chloride by introduction of sulfur dioxide into the reaction in the presence of copper (I) chloride.

The groups R^3 and R^4 connected to Z can be further derivatized. For example, when R^3 or R^4 is an ester of a carboxylic acid or alcohol the compound can be transformed into the respective carboxylic acid or alcohol analog using standard conditions. The conditions to effect these transformations include aqueous base in which one or more molar equivalents of alkali metal hydroxide such as sodium hydroxide is used in water with a co-solvent such as THF, dioxane or a lower alcohol such as methanol or mixtures of THF and a lower alcohol at temperatures ranging from 0° C. to 40° C. When R^3 or R^4 is a carboxylic acid or ester the compound can be reduced to the respective primary alcohol analog using standard conditions such as lithium aluminum hydride in ethyl ether. When R^3 or R^4 is an aldehyde or ketone the compound can be reduced to the respective primary alcohol analog using a metal catalyst, by sodium in alcohol, sodium borohydride and by lithium aluminum hydride. When R^3 or R^4 is an ether, the compound can be transformed to the free alcohol by using one to ten molar equivalents of a strong Lewis acid such as a trihaloborane, most conveniently tribromoborane in a halocarbon solvent such as dichloromethane. When R^3 or R^4 is an alcohol the, compound can be oxidized to the respective aldehyde, carboxylic acid or ketone analog using a transition metal oxidant (chromium trioxide-pyridine, pyridinium chlorochromate, manganese dioxide) in an inert solvent such as ether, dichloromethane. Alcohols can also be oxidized using DMSO with a number of electrophilic molecules (dicyclohcxylcarbodiimide, acetic anhydride, trifluoro acetic anhydride, oxalyl chloride and sulfur dioxide). When R^3 or R^4 is a carboxylic acid the compound can be transformed into a carboxylic acid amide analog. This transformation can be accomplished using standard methods to effect carboxylic acid to carboxylic acid amide transformations. These methods include converting the acid to an activated acid and reacting with one or more molar equivalents of the desired amine. Amines in this category include ammonia in the form of ammonium hydroxide, hydroxyl amine and 2-aminopropionitrile. Methods to activate the carboxylic acid include reacting said acid with one or more molar equivalents of oxalyl chloride or thionyl chloride to afford the carboxylic acid chloride in a suitable solvent such as dichloromethane, chloroform or diethyl ether. This reaction is often catalyzed by adding small amounts (0.01 to 0.1 molar equivalents) of dimethylformamide. Other methods to activate the carboxylic acid include reacting said acid with one or more molar equivalents dicyclohexylcarbodiimide with or without one or more molar equivalents of hydroxybenzotriazole in a suitable solvent such as dichloromethane or dimethylformamide at temperatures ranging from 0° C. to 60° C. When R^3 or R^4 is nitro, the compound can be reduced to the respective amino compound most readily using tin dichloride in ethylacetate at 40 to 100° C. or with hydrazine and Montmorillnite clay in ethanol at 40 to 100° C. or by catalytic hydrogenation in the presence of a catalyst such as palladium on carbon. When R^3 or R^4 is an amino or an alcohol, the compound can be acylated using one or more molar equivalents of suitable acylating agent. The acylating agent is generally a lower alkyl or aryl carboxylic acid anhydride or a lower alkyl or aryl carboxylic acid chloride. The reaction is run under standard conditions, for example the use of pyridine as solvent with or without a co-solvent such as dichloromethane at 0° C. to room temperature. When R^3 or R^4 is an alcohol it can be acylated with a lower alkyl or aryl carboxylic acid anhydride in the presence of magnesium iodide in diethyl ether at ambient temperature to reflux. When R^3 or R^4 is a nitrile it can be reduced to the aminoalkyl compound by tin (II) chloride in refluxing ethyl acetate or by catalytic hydrogenation in the presence of a catalyst such as Raney nickel or by lithium aluminum hydride in an inert solvent such as ether. When R^3 or R^4 is a nitrile it can be converted to a carboxylic acid amide using standard conditions such as HCl/H_2O at ambient temperatures to reflux or a milder procedure involves the reaction of the nitrile with an alkaline solution of hydrogen peroxide. When R^3 or R^4 is halogen or trifluoromethanesulfonate it can be converted to a 3-hydroxy-cyclobut-3-ene-4-yl-1,2-dione by methodology of Liebeskind et. al. (*J. Org. Chem.* 1990, 55, 5359). When R^3 or R^4 is an alcohol can be alkylated with a suitable alkylating agent such as one or more molar equivalents of alkyl halide in the presence a base such as potassium carbonate or sodium hydroxide in a suitable solvent such as TBF, DMF or DMSO at temperatures ranging from 0° C. to 60° C. When R^3 or R^4 is a carboxylic acid, the compound can be coupled to tetronic acid with a coupling reagent such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in the presence

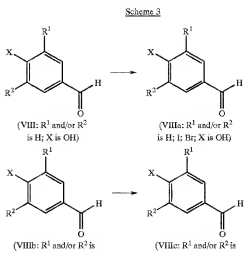
of a base such as triethylamine or DMAP in a suitable solvent such as DMF.

Scheme 2



The following method will prepare derivatives of formula (V: Y is methylene) that can utilized in Scheme 1 to prepare compounds of formula (I: Y is methylene). The ketone (V: Y is carbonyl) can be reduced with lithium aluminum hydride in an aprotic solvent such as THF at ambient temperature to give the alcobol (VII). Further reduction of alcobol (VII) with triethylsilane in the presence of boron trifluoride dietlayl etherate provides the methylene compound (V: Y is methylene). The compounds prepared in Scheme 2 of formula (V: Y is methylene) can be further modified synthetically in Scheme 5.

Scheme 3



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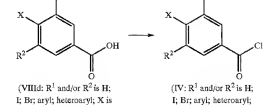


Derivatives of formula (IV: R^1 and/or R^2 is H; I; Br; aryl; heteroaryl; X is O-alkyl) can be prepared according to Scheme 3. The p-hydroxybenzaldehyde (VIII) can be conveniently iodinated to the diiodophenol of formula (VIIIa: R^1 and R^2 is I) using at least two molar equivalents of iodine in the presence of two or more molar equivalents of an alkali metal hydroxide such as NaOH in an alcobol solvent such as methanol at −20° C. to room temperature. Similarly the monoiodophenol (VIIIa: R^1 is H; R^2 is I) can be prepared from the phenol of formula (VIII) using one to 1.5 molar equivalents of iodine in the presence of at least one equivalent of an alkali metal hydroxide such as NaOH in a alcobol solvent such as methanol at −20° C. to room temperature. Either the monoiodophenol (VIIIa: R^1 is H; R^2 is I) or the diiodophenol (VIIIa: R^1 and R^2 is I) can be converted to the respective alkyl ether derivatives of formula (VIIIb: R^1 is H; R^2 is I; X is —O-alkyl) or (VIIIb: R^1 and R^2 is I; X is —O-alkyl) by reacting the phenol moicty with a suitable alkylating agent such as one or more molar equivalents of methyl iodide or dimethylsulfate employing a base such an alkali methyl carbonate or hydroxide such as potassium carbonate or sodium hydroxide in a suitable solvent such as THF, DMFN or DMSO. The reaction is generally performed at temperatures ranging from 0° C. to 60° C. The mono or dibrominated benzaldehydes of formula (VIIIb: R^1 and/or R^2 is Br; X is —O-alkyl) can be prepared in analogs fashion by substituting bromine for iodine in the sequence above.

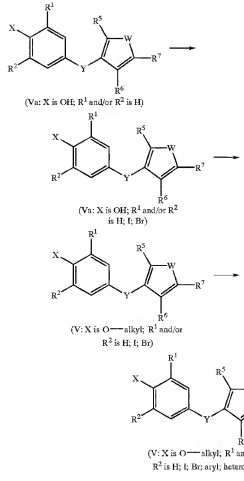
The mono or diiodo alkyl ether benzaldehydes of formula (VIIIb: R^1 and/or R^2 is I; X is O-alkyl) can be reacted with an arylboronic acid or heteroarylboronic acid to afford the product of formula (VIIIc: R^3 and/or R^2 is aryl or heteroaryl; X is —O-alkyl) under the conditions of the Suzuki Reaction (*Journal of the Chemical Society Chemical Communications* 1979 886 and *Synthetic Communications* 1981 11(7) 513). The other co-reagents necessary to effect the Suzuki Reaction include one or more molar equivalents of a metal catalyst such as tetrakis(triphenylphosphine)palladium or palladium (II) acetate and a base such as barium hydroxide octahydrate or sodium carbonate in a solvent such as benzene, toluene or DME/H_2O. The starting aryl or heteroaryl boronic acids are commercially available or can be prepared by standard synthetic methods.

The mono or diaryl or mono or diheteroaryl benzaldehyde analogs of formula (VIIIc: R^2 and/or R^2 is aryl or heteroaryl; X is —O-alkyl) can be converted to the corresponding mono or diaryl or mono or diheteroaryl benzoic acid analogs of formula (VIIId: R^1 and/or $R^2$is aryl or heteroaryl; X is —O-alkyl) using the oxidative conditions of silver (I) oxide in an aqueous base such as sodium hydroxide at temperatures ranging from 50° C. to reflux.

The benzoic acid compound (VIIId: R^1 and/or R^2 is H; I; Br; aryl; heteroaryl; X is O-alkyl) can be converted to the corresponding benzoic acid chloride (IV: R^1 and/or R^2 is H; I; Br; aryl heteroaryl; X is O-alkyl) by standard procedures using reagents such as oxalyl chloride and thionyl chloride. The compounds prepared in Scheme 3 of formula (IV: R^1 and/or R^2 is H; I; Br, aryl; heteroaryl; X is O-alkyl) can be

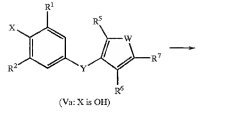
utilized in Scheme-$_1$ to prepared compounds of formula (I: R^1 and/or R^2 is H; I; Br; aryl; heteroaryl).

Scheme 4



In an analogous synthetic sequence to Scheme 3, compounds of formula (Va: X is OH; R^1 and/or R^2 is H) can be functionalized at positions R^1 and R^2 to give compounds of formula (V: X is O-alkyl; R^1 and/or R^2 is H; I; Br; aryl; heteroaryl). The compounds prepared in Scheme 4 of formula (V: R^1 and/or R^2 is H; I; Br; aryl; heteroaryl; X is O-alkyl) can be utilized in Scheme 1 to prepared compounds of formula (I: R^1 and/or R^2 is H; I; Br; aryl; heteroaryl). The compounds prepared in Scheme 4 of formula (V: R^1 and/or R^2 is H; I; Br; aryl; heteroaryl; X is O-alkyl) can be synthetically modified in Scheme 5.

Scheme 5



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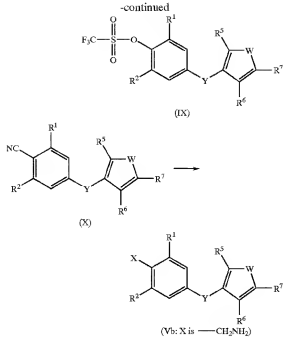
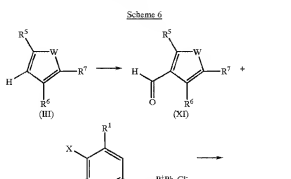


In a three step process (Scheme 5) compounds of formula (Va: X is OH) can be converted to compounds of formula (Vb: X is $—CH_2NH_2$). Reaction of compounds of formula (Va: X is OH) with trifluoromethanesulfonic anhydride or trifluoromethanesulfonic acid chloride in the presence of a organic base such as pyridine or triethylamine in dichloromethane at 0° C. to ambient temperature provides compound (IX). The triflate (IX) can be converted to the carbonitrile (X) with potassium cyanide or zinc cyanide in the presence of tetrakistriphenylphosphinenickel(0) which can be generated in situ from bistriphenylphosphinenickel (II) bromide and ZnPPh$_3$. The nitrile (X) can be reduced to the aminoalkyl compound (Vb: X is $—CH_2NH_2$) by tin (II) chloride in refluxing ethyl acetate or by catalytic hydrogenation in the presence of a catalyst such as Raney nickel or by lithium aluminum hydride in an inert solvent such as ether.

From Scheme 5, the prepared compounds of formula (Vb: X is $—CH_2NH_2$) can be used in Scheme 1 to prepared sulfonamides of formula (I: X is $—CH_2NH—$).

Scheme 6



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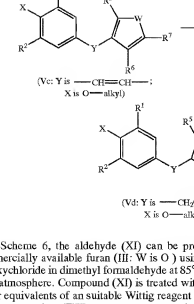
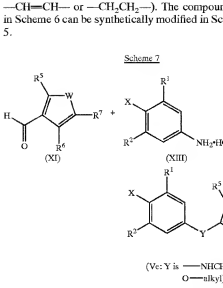


In Scheme 6, the aldehyde (XI) can be prepared from commercially available furan (III: W is O) using phosphorus oxychloride in dimethyl formaldehyde at 85° C. under an inert atmosphere. Compound (XI) is treated with one to 1.3 molar equivalents of an suitable Wittig reagent in a nonprotic solvent such as THF at temperatures ranging from −78° C. to room temperature under an inert atmosphere such as nitrogen or argon to provide the olefin derivative. The olefin (Vc: Y is —CH═CH—) can be converted to the alkane (Vd: Y is —CH_2CH_2—) through any standard procedure for hydrogenation. The most convenient method of reduction is catalytic hydrogenation employing 10% palladium on carbon over an atmosphere of hydrogen for 12–24 hours.

The compounds prepared in Scheme 6 of formula (Vc: Y is —CH═CH—) or (Vd: Y is —CH2CH2—) can be utilized in Scheme 1 to prepared compounds of formula (I: Y is —CH═CH— or —CH_2CH_2—). The compounds prepared in Scheme 6 can be synthetically modified in Schemes 4 and 5.

Scheme 7



Compounds of formula (Vc: Y is —NHCH2—) in Scheme 7 can be prepared through a number of reductive amination procedures such as the method of Maryanoff, et. al. (*J. Org. Chem.* 1996, 61, 3849–62), but is easily prepared by a modified procedure of Borch, et. al. (*J. Am. Chem. Soc.* 1971, 93, 2897–04. A solution of the aldehyde (XI) (as prepared in Scheme 6), and the appropriate aniline hydrochloride (XIII) (1.2–1.5 equivalent) in a suitable protic solvent such as methanol is stirred at room temperature in the presence of sodium cyanoborohydride (1.1–1.5 equivalent) yields compounds of formula (Ve: Y is —NHCH2—).

The compounds prepared in Scheme 7 of formula (Ve: Y is —NHCH2—) can be utilized in Scheme 1 to prepared compounds of formula (I: Y is —NHCH2—). The compounds prepared in Scheme 6 can be synthetically modified in Schemes 4 and 5.

The compounds of this invention are useful in treating metabolic disorders related to insulin resistance or hyperglycemia, typically associated with obesity or glucose intolerance. The compounds of this invention are therefore, particularly useful in the treatment or inhibition of type II diabetes. The compounds of this invention are also useful in modulating glucose levels in disorders such as type I diabetes.

The ability of compounds of this invention to treat or inhibit disorders related to insulin resistance or hyperglycemia was established with representative compounds of this invention in the following standard pharmacological test procedure which measures the inhibition of PTPase.

Inhibition of Tris-Phosphorylated Insulin Receptor Dodecaphosphopeptide Dephosphorylation by hPTP 1B

This standard pharmacological test procedure assess the inhibition of recombinant rat protein tyrosine phosphatase, PTP1B, activity using, as substrate, the phosphotyrosyl dodecapeptide corresponding to the 1142–1153 insulin receptor kinase domain, phosphorylated on the 1146, 1150 and 1151 tyrosine residues. The procedure used and results obtained are briefly described below.

Human recombinant PTP1B was prepared as described by Goldstein (see Goldstein et al. *Mol. Cell Biochem.* 109, 107, 1992). The enzyme preparation used was in microtubes containing 500–700 μg/ml protein in 33 mM Tris-HCl, 2 mM EDTA, 10% glycerol and 10 mM 2-mercaptoethanol. Measurement of PTPase activity.

The malachite green-ammonium molybdate method, as described (Lanzetta et al. *Anal. Biochem.* 100, 95, 1979) and adapted for a platereader, is used for the nanomolar detection of liberated phosphate by recombinant PTP1B. The test procedure uses, as substrate, a dodecaphosphopeptide custom synthesized by AnaSpec, Inc. (San Jose, Calif.), the peptide, TRDIYETDYYRK, corresponding to the 1142–1153 catalytic domain of the insulin receptor, is tyrosine phosphorylated on the 1146, 1150, and 1151 tyrosine residues. The recombinant rPTP1B is diluted with buffer (pH 7.4, containing 33 mM Tris-HCl, 2 mM EDTA and 50 mM b-mercaptoethanol) to obtain an approximate activity of 1000–2000 nmoles/min/mg protein. The diluted enzyme (83.25 mL) is preincubated for 10 min at 37° C. with or without test compound (6.25 mL) and 305.51 mL of the 81.83 mM HEPES reaction buffer, pH 7.4 peptide substrate, 10.5 ml at a final concentration of 50 mM, and is equilibrated to 37° C. in a LABLINE Multi-Blok heater equipped with a titerplate adapter. The preincubated recombinant enzyme preparation (39.5 ml) with or without drug is added to initiate the dephosphorylation reaction, which proceeds at 37° C. for 30 min. The reaction is terminated by the addition of 200 mL of the malachite green-ammonium molybdate-Tween 20 stopping reagent (MG/AM/Tw). The stopping reagent consists of 3 parts 0.45% malachite green hydrochloride, 1 part 4.2% ammonium molybdate tetrahydrate in 4 N HCl and 0.5% Tween 20. Sample blanks are prepared by the addition of 200 mL MG/AMJFw to substrate

and followed by 39.5 ml of the preincubated recombinant enzyme with or without drug. The color is allowed to develop at room temperature for 30 min. and the sample absorbances are determined at 650 nm using a platereader (Molecular Devices). Sample and blanks are prepared in quadruplicates.

Calculations:

PTPase activities, based on a potassium phosphate standard curve, are expresses as nmoles of phosphate released/min/mg protein, inhibition of recombinant PTP1B by test compounds is calculated as percent of phosphatase control. A four parameter non-linear logistic regression of PTPase activities using SAS release 6.08, PROC NLIN, is used for determining IC_{50} values of test compounds. The following results were obtained.

Example	IC50 (μM)
1	0.251
2	0.130
3	0.421
4	0.559
5	0.083
6	0.256
7	0.180
8	0.075
9	0.083
10	0.226
11	0.099
12	0.183
13	0.267
14	0.060
15	28.78% inhibition @ 1.0 μM
16	22.81% inhibition @ 0.5 μM
17	0.643
18	0.404
19	0.633
20	0.148
21	0.371
22	0.508
23	0.439
24	0.254
25	0.241
26	51.37% inhibition @ 1.0 μM
27	22.99% inhibition @ 1.0 μM
28	0.577
Phenylarsine oxide (reference standard)	39.7
Sodium orthovanadate (reference standard)	244.8
Ammonium molybdate tetrahydrate (reference standard)	8.7

Based on the results obtained in the standard pharmacological test procedure, representative compounds of this invention have been shown to inhibit PTPase activity and are therefore useful in treating metabolic disorders related to insulin resistance or hyperglycemia, typically associated with obesity or glucose intolerance. More is invention useful in the treatment or inhibition of type II diabetes, and in modulating glucose levels in disorders such as type I diabetes. As used herein, the term modulating means maintaining glucose levels within clinically normal ranges.

Effective administration of these compounds may be given at a daily dosage of from about 1 mg/kg to about 250 mg/kg, and may given in a single dose or in two or more divided doses. Such doses may be administered in any manner useful in directing the active compounds herein to the recipient's bloodstream, including orally, via implants, parenterally (including intravenous, intraperitoncal and subcutaneous injections), rectally, vaginally, and transdermally. For the purposes of this disclosure, transdermal administrations are understood to include all administrations across the surface of the body and the inner linings of bodily passages including epithelial and mucosal tissues. Such administrations may be carried out using the present compounds, or pharmaceutically acceptable salts thereof, in lotions, creams, foams, patches, suspensions, solutions, and suppositories (rectal and vaginal).

Oral formulations containing the active compounds of this invention may comprise any conventionally used oral forms, including tablets, capsules, buccal forms, troches, lozenges and oral liquids, suspensions or solutions. Capsules may contain mixtures of the active compound(s) with inert fillers and/or diluents such as the pharmaceutically acceptable starches (e.g. corn, potato or tapioca starch), sugars, artificial sweetening agents, powdered celluloses, such as crystalline and microcrystalline celluloses, flours, gelatins, gums, etc. Useful tablet formulations may be made by conventional compression, wet granulation or dry granulation methods and utilize pharmaceutically acceptable diluents, binding agents, lubricants, disintegrants, suspending or stabilizing agents, including, but not limited to, magnesium stcarate, stearic acid, talc, sodium lauryl sulfate, microcrystalline cellulose, carboxymethylcellulose calcium, polyvinylpyrrolidone, gelatin, alginic acid, acacia gum, xanthan gum, sodium citrate, complex silicates, calcium carbonate, glycine, dextrin, sucrose, sorbitol, dicalcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, talc, dry starches and powdered sugar. Oral formulations herein may utilize standard delay or time release formulations to alter the absorption of the active compound(s). Suppository formulations may be made from traditional materials, including cocoa butter, with or without the addition of waxes to alter the suppository's melting point, and glycerin. Water soluble suppository bases, such as polyethylene glycols of various molecular weights, may also be used.

It is understood that the dosage, regimen and mode of administration of these compounds will vary according to the malady and the individual being treated and will be subject to the judgment of the medical practitioner involved. It is preferred that the administration of one or more of the compounds herein begin at a low dose and be increased until the desired effects are achieved.

The following procedures describe the preparation of representative examples of this invention.

EXAMPLE 1

4-[2,6-Dibromo-4-(2-ethyl-benzofuran-3-carbonyl)-phenoxysulfonyl]-2-hydroxy-benzoic acid

Step 1

4-Chlorosulphonyl-2-hydroxybenzoic acid

At ambient temperature, to a stirred solution of commercial 4-aminosalicylic acid sodium salt dihydrate (50.25 g, 0.2379 mol) in H_2O (119 mL) was added a solution of 10% aq. NaOH (3.40 mL) and sodium nitrite (18.06 g, 0.2617 mol) in H_2O (44 mL). This solution was added to a vigorously stirred mixture of conc. HCL (153 mL) and glacial HOAc (76 mL) while maintaining the reaction temperature at −10 ° C. After 5 min., the dark orange suspension was added to a vigorously stirred mixture of copper (I) chloride (2.355 g, 0.02379 mol) in HOAc (128 mL) which had been previously cooled to 0° C. The reaction was saturated with sulfur dioxide for 0.5 h. The ice bath was removed and the reaction was stirred for 18 h. The reaction was quenched into crushed ice (2 L), allowed to warm to ambient temperature and filtered. The crude product was slurried in 20% THF/ether (1 L), dried ($MgSO_4$), filtered and concentrated to give

36.32 g (64%) of the title compound as a red solid, mp 170–185° C.; ^{1}H NMR (DMSO-d6) δ7.11–7.16 (m, 2 H), 7.76 (d, 1 H), 13.2–14.4 (br. s, 2 H).

Step 2

4-[2,6-Dibromo4-(2-ethyl-benzofuran-3-carbonyl)-phenoxvsulfonvll-2-hydroxy-benzoic acid

At ambient temperature, to a stirred solution of commercial benzbromarone (0.243 g, 0.574 mmol) in 0.5M aq. $NaHCO_3$:THF (8 mL; 1:1) was added portionwise 4-chlorosulphonyl-2-hydroxybenzoic acid (0.271 g, 1.15 mmol) while maintaining the pH at 8 with the simultaneous addition of 0.5M aq. $NaHCO_3$. After the addition was complete the reaction was stirred for 18 h. To the reaction was added 4-chlorosulphonyl-2-hydroxybenzoic acid (0.271 g, 1.15 mmol) while maintaining the pH at 8 with the simultaneous addition of 0.5M aq. $NaHCO_3$. After 2 h, the reaction was quenched with 2N HCl (25 mL), extracted with EtOAc and concentrated. The crude product was purified by preparative HPLC (C18, eluting with 75% CH_3CN/H20 containing 0.1% TFA) and crysallized from EtOAc/hexane to give 0.14 g (39%) of the title compound as a white solid, mp 173° C.; ^{1}H NMR (DMSO-d6) δ1.25 (t, 3 H), 2.76 (q, 2 H), 7.30 (dt, 1 H), 7.36 (dt, 1 H), 7.44–7.49 (m, 3 H), 7.66 (d, 1 H), 8.03 (d, 1 H), 8.08 (s, 2 H). IR (KBr) 3400, 2950, 1680, 1650, 1400 and 1180 cm^{-1}. mass spectrum (–ESI), m/z 621/623/625 (M–H). Anal. Calcd. for $C_{24}H_{16}Br_2O_8S$: C, 46.18; H, 2.58; N, 0.00. Found: C, 46.41; H, 2.64; N, 0.00.

EXAMPLE 2

4-[5'-(2-Butyl-benzofuran-3-carbonyl)-[1,1'; 3'1"] terphenyl-2'-yloxysulfonyl]-2-hydroxy-benzoic acid

Step 1

2-n-Butyl-3-(4-hydroxy-3.5-diiodobenzoyl)benzofuran

At 0° C., to a stirred solution containing commercial 2-n-butyl-3-(4-hydroxybenzoyl)benzofuran (1.05 g, 3.57 mmol) and sodium hydroxide (0.286 g, 7.14 mmol) in MeOH (35.7 mL) was added iodine (2.27 g, 8.93 mmol). After 24h, the reaction was quenched with 1N HCl (30 mL) and extracted with ether. The combined ethereal extracts were washed with 10% aq. $Na_2S_2O_3$ (3×), with brine (3×), dried ($MgSO_4$) and concentrated. The crude product was purified on Biotage KP-Sil to give 0.914 g (47%) of the title compound. ^{1}H NMR (DMSO-d6) δ0.85 (t, 3 H), 1.27 (sextet, 2 H), 1.71 (quintet, 2 H), 2.77 (t, 2 H), 7.29–7.36 (m, 2 H), 7.45 (d, 1 H), 7.65 (d, 1 H), 8.11 (s, 2 H), 10.5 (br s, 1 H).

Step 2

2-n-Butyl-3-(4-[(2-methoxethoxy)methoxy]-3.5-diiodobenzoyl)benzofuran

At 0° C., to a stirred solution of 2-n-butyl-3-(4-hydroxy-3,5-diiodobenzoyl)benzofuran (0.897 g, 1.64 mmol) in THF (16.4 m) was added 60% NaH/mineral oil (85.4 mg, 2.14 mmol). After 0.5 h, to the reaction was added MEM chloride (0.301 mL, 2.63 mmol) and the reaction was stirred for 18 h eventually warming to ambient temperature. The reaction was quenched with 1N NaOH (30 mL) and extracted with ether. The ethereal extracts were washed with 1N NaOH (3×), with brine (3×), dried (K_2CO_3) and concentrated to give 1.042 g (100%) of the title compound. ^{1}H NMR (DMSO-d6) δ0.85 (t, 3 H), 1.26 (sextet, 2 H), 1.70 (quintet, 2 H), 2.74 (t, 2 H), 3.28 (s, 3 H), 3.56 (t, 2 H), 4.03 (t, 2 H), 5.26 (s, 2 H), 7.28–7.40 (m, 2 H), 7.49 (d, 1 H), 7.66 (d, 1 H), 8.18 (s, 2 H).

Step 3

(2-n-Butyl-benzofuran-3-yl)-(2'-[(2-methoxethoxy) methoxy]-[1,1'; 3',1"]terphenyl-5'-yl)-methanone

At ambient temperature, to a stirred mixture containing phenylboronic acid (0.454 g, 3.73 mmol), barium hydroxide octahydrate (1.60 g, 5.08 mmol) and palladium (II) acetate (7.60 mg, 0.0339 mmol) in DME:H_2O (6:1, 20 mL) was added a solution of 2-n-butyl-3-(4-[(2-methoxyethoxy) methoxy]-3,5-diiodobenzoyl)benzofuran (1.07 g, 1.69 mmol) in DME:H_2O (6:1, 20 mL). After the addition was complete, the reaction was heated at 80° C. for 16h. The reaction was cooled to ambient temperature, diluted with ether (50 mL), washed sequentially with sat. aq. $NaHCO_3$ (3×), with brine (3×), dried ($MgSO_4$) and concentrated. The crude product was purified on Biotage KP-Sil eluting with 25% ether/pet. ether to give 0.380 g (42%) of the title compound. ^{1}H NMR (DMSO-d6) δ0.76 (t, 3 H), 1.22 (sextet, 2 H), 1.64 (quintet, 2 H), 2.81–2.92 (m, 6 H), 3.03 (s, 3 H), 4.44 (s, 2 H), 7.31–7.67 (m, 14 H), 7.76 (s, 2 H).

Step 4

(2-n-Butyl-benzofuran-3-yl)-(2'-hydroxy-[1,1: 3',1"] terphenyl-5'-yl)-methanone

At ambient temperature, to a stirred solution of (2-n-butyl-benzofuran-3-yl)-(2'-[(2-methoxyethoxy)methoxy]-[1,1';3',1"]terphenyl-5'-yl)-methanone (0.371 g, 0.695 mmol) in CH_2Cl_2 (3.71 mL) was added trifluoroacetic acid (3.71 mL). After 0.5h, the reaction was diluted with ether (50 mL), washed with H20 (3×), dried ($MgSO_4$) and concentrated. The crude product was purified on Biotage KP-Sil eluting with 10% acetone/hexane to give 0.281 g (90%) of the title compound. ^{1}H NM (DMSO-d6) δ0.77 (t, 3 H), 1.22 (sextet, 2 H), 1.64 (quintet, 2 H), 2.87 (t, 2 H), 7.29–7.65 (m, 14 H), 7.66 (s, 2 H), 9.39 (s, 1 H).

Step 5

4-[5'-(2-Butyl-benzofuran-3-carbonyl)-[1,1'; 3'1"]terphenyl-2'-yloxysulfonyl]-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 1, step 2, using (2-n-butyl-benzofuran-3-yl)-(2'-hydroxy-[1,1';3 ',1"]terphenyl-5'-yl)-methanone (0.136 g, 0.305 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.144 g, 6.09 mmol) to give 0.080 g (38%) of the title compound as a white solid, mp 190–197° C.; ^{1}H NMR (DMSO-d6) δ0.77 (t, 3 H), 1.22 (sextet, 2 H), 1.63 (quintet, 2 H), 2.83 (t, 2 H), 6.55 (d, 1 H), 6.66 (dd, 1 H), 7.28–7.38 (m, 8 H), 7.41-7.43 (m, 4 H), 7.51 (dd, 1 H), 7.57 (d, 1 H), 7.64 (d, 1 H), 7.74 (s, 2 H). IR (KBr) 2900, 1675, 1390 and 1190 cm^{-1}. mass spectrum (–ESI), mlz 645 (M–H). Anal. Calcd. for $C_{39}H_{30}O_8S.2H_2O$: C, 66.85; H, 5.02; N, 0.00. Found: C, 66.80; H, 4.88; N.0.07.

EXAMPLE 3

4-[4-(2-Ethyl-benzofuran-3-carbonyl)-2,6-dimethyl-phenoxysulfonyl]-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 1, step 2, using 2-n-ethyl-3-(4-hydroxy-3,5-dimethylbenzoyl)benzofuran (0.250 g, 0.849 mmol, RN 52489-58-4) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.402 g, 1.70 mmol) to give 0.15 g (36%) of the title compound as a white solid, mp 166–167° C.; ^{1}H NMR (DMSO-d6) δ1.24 (t, 3 H), 2.14 (s, 6 H), 2.74 (q, 2 H), 7.30 (dt, 1 H), 7.36 (dt, 1 H), 7.44–7.48 (m, 3 H), 7.57 (s, 2 H), 7.66 (d, 1 H), 8.05 (d, 1 H). IR (KBr) 3400, 2950, 1680, 1650 and 1180 cm^{-1}. mass spectrum (–ESI), m/z 493 (M–H). Anal. Calcd. for $C_{26}H_{22}O_8S.0.4H_2O$: C, 62.24; H, 4.58; N, 0.00. Found: C, 62.16; H, 4.53; N, 0.10.

EXAMPLE 4

4-[4-(2-Ethyl-benzofuran-3-carbonyl)-2,6-diisopropyl-phenoxysulfonyl]-2-hydroxy-benzoic acid

At 5° C., to a stirred suspension containing 2-n-ethyl-3-(4-hydroxy-3,5-diisopropylbenzoyl)benzofuran (0.300 g,

0.856 mmol, RN 52901-28-7) and 0.05M Tris buffer pH 9 (3.41 mL) in THF (1.03 mL) was added dropwise, a solution of 4-chlorosulphonyl-2-hydroxybenzoic acid (0.243 g, 1.03 mmol) in THF (2.05 mL) while maintaining the pH at 10 with the simultaneous addition of 2N NaOH. After the addition was complete, the reaction was stirred for 1.5h. To the reaction was added 4-chlorosulphonyl-2-hydroxyhenzoic acid (0.243 g, 1.03 mmol) in THF (2.05 mL) while maintaining the pH at 10 with the simultaneous addition of 2N NaOH. and the reaction was stirred for 1.5h. The reaction was quenched with 2N HCl (40 mL) and extracted with EtOAc. The combined organic extracts were washed with 2N HCl (3×), dried ($MgSO_4$) and concentrated. The crude product was purified hy preparative HPLC (C18, eluting with 75% CH_3CN/H_2O containing 0.1% TFA) to give 0.30 g (64%) of the title compound as a white solid, mp 200–201° C.; ^1H NMR (DMSO-d6)δ1.05 (d, 12 H), 1.24 (t, 3 H), 2.80 (q, 2 H), 3.09 (septet, 2 H), 7.26 (dt, 1 H), 7.32–7.36 (m, 2 H), 7.45 (d, 1 H). 7.49 (dd, 1 H), 7.62 (s, 2 H), 7.64–7.66 (m, 1 H), 8.06 (d, 1 H).IR (KBr) 3400, 2950, 1680, 1620, 1490 and 1190 cm^{-1}. mass spectrum (EI), m/z 550 (M +). Anal. Calcd. for $C_{36}H_{30}O_8S.0.8H_2O$: C, 63.77; H. 5.64; N, 0.00. Found: C, 63.81; H, 5.55; N, 0.10.

EXAMPLE 5

4-[4-(2-Benzyl-4,5-dimethyl-furan-3-carbonyl)-2,6-diisopropyl-phenoxysulfonyl]-2-hydroxy-henzoic acid

Step 1

2-Benzyl-4.5-dimethylfuran

At −78° C., to a stirred solution of commercial 2,3-dimethylfuran (50 g, 0.520 mol) in THF (2.6 L) was add dropwise 1.6M n-BuLihexanes (325 mL, 0.520 mol). After the addition was complete, the dry ice/acetone bath was removed and the reaction was stirred for 1h. At −78° C., to the reaction was added dropwise commercial henzyl bromide (62 mL, 0.520 mol). After the addition was complete the reaction was stirred at −78° C. for 6h, the dry ice/acetone bath was removed and the reaction was stirred for 7 days. The reaction was concentrated in vacuo and purified on silica gel eluting with hexane to give 75.70 g (78%) of the title compound as a clear oil; ^1H NMR (DMSO-d6) δ1.83 (s, 3 H), 2.08 (s, 3 H), 3.83 (s, 2 H), 5.83 (s, 1 H), 7.16–7.30 (m, 5 H). mass spectrum (EI), m/z 186 (M+).

Step 2

(2-Benzyl-4,5-dimethyl-furan-3-yl)-(3,5-diisopropyl4-methoxy-phenyl)-methanone

At ambient temperature, to a stirred suspension containing 3,5-diisopropyl-p-anisic acid (1.95 g, 8.23 mmol, RN-1 17439-59-5) and oxalyl chloride (0.8 mL, 9.20 mmol) in CH_2Cl_2 (22.5 mL) was added N,N-dirnethylformamide (2 drops). After 2h, the reaction was cooled to −78° C. To the reaction was added tin (TV) chloride (1.05 mL) followed by a solution of 2-henzyl4,5-dimethylfuran (1.8 g, 9.68 mmol) in CH_2Cl_2 (10.0 mL). After the additions were complete, the reaction was allowed to warm to ambient temperature and stirred for 24 h. The reaction was quenched into crushed ice (100 g), diluted with sat. aq. KH_2PO_4 (100 mL) and extracted with ether. The combined ethereal extracts were washed with sat aq. $NaHCO_3$ (2×60 mL), with brine (1×60 ml), dried (Na_2SO_4) and concentrated. The crude product was purified on Biotage KP-Sil eluting with 1% EtOAc/pet. ether to give 2.07 g, (62%) of the title compound as an oil; ^1H NMR (DMSO-d6)δ7.1 17 (d, 12 H), 1.81 (s, 3 H), 2.19 (s, 3 H), 3.29 (septet, 2 H), 3.74 (s, 3 H), 3.84 (s, 2 H), 7.01–7.03 (m, 2 H), 7.16–7.25 (m, 3 H), 7.47 (s,2H). mass spectrum (EI), m/z 404 (M+). Anal. Calcd. for $C_{27}H_{32}O_3$ .0.6H_2O: C, 78.07; H, 8.06; N, 0.00. Found: C, 78.04; H, 7.93; N, −0.02.

Step 3

(2-Benzyl4.5-dimethyl-furan-3-yl)-(3 .5-diisopropyl-4-hydroxy-phenyl)-methanone

At −78° C., to a stirred solution of (2-benzyl-4,5-dimethyl-furan-3-yl)-(3,5-diisopropyl-4-methoxy-phenyl)-methanone (2.00 g, 4.94 mmol) in CH_2Cl_2 (16.6 mL) was added 1M boron trihromide/CH_2Cl_2 (10.4 mL). After the addition was complete, the dry ice/acetone bath was replaced with an ice bath and the reaction was stirred for 1.5 h. The reaction was quenched into crushed ice (18 g), diluted with H_2O(20 mL) and extracted with ether. The combined ethereal extracts were washed with brine, dried ($MgSO_4$) and concentrated. The crude product was purified on silica gel eluting with 15% acetone/hexane to give 0.97 g (50%) of the titde compound as a white solid, mp 138° C.; ^1H NMR (DMSO-d6) δ1.14 (d, 12 H), 1.80 (s, 3 H), 2.19 (s, 3 H), 3.34 [septct (under H_2O peak), 2 H], 3.84 (s, 2 H), 7.04 (d, 2 H), 7.17–7.25 (m, 3 H) 7.44 (s, 2 H), 9.11 (s, 1 H). IR (KBr) 3400, 2950, 1580, 1320, 1200 cm^{-1}. mass spectrum (EI), m/z 390 (M +). Anal. Calcd. for $C_{26}H_{30}O_3$. 0.2H_2O: C, 79.24; H, 7.77; N, 0.12. Found: C, 79.27; H, 7.85; N, 0.12.

Step 4

4-[4-(2-Benzyl-4,5-dimethyl-furan-3-carhonyl)-2,6-diisolpropyl-phenoxysulfonul]2-hydroxy-henzoic acid

The title compound was prepared according to the procedure in Example 4 using (2-benzyl-4,5-dimethyl-furan-3-yl)-(3,5-diisopropyl-4-hydroxy-phenyl)-methanone (0.300 g, 0.768 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.218 g, 0.922 mmol) to give 0.04 g (9%) of the title compounnd as a tan solid, mp 142–143° C.; ^1H NMR (DMSO-d6) δ1.04 (d, 12 H), 1.81 (s, 3 H), 2.19 (s, 3 H), 3.07 (septet, 2 H), 3.81 (s, 2 H), 6.95–6.97 (m, 2 H), 7.14–7.20 (m, 3 H), 7.40–7.46 (m, 2 (s, 2 H), 8.03 (d, 1 H). IR (KBr) 3400, 2950, 1675, 1375, 1180 cm^{-1}. mass spectrum (EI), m/z 590 (M+). Anal. Calcd. for $C_{33}H_{34}O_8S$. 1.1H_2O: C, 64.92; H, 5.98; N, 0.00. Found: C, 64.92; H, 5.76; N, −0.03.

EXAMPLE 6

4-[5'-(2-Ethyl-benzofuran-3-carhonyl)-[1,1: 3',1"] terphenyl-2'-yloxysulfonyl-]2-hydroxy-benzoic acid sodium salt

Step 1

3,5-Diiodo-4-(2-methoxyethoxymethoxy)henzaldehyde

The title compound was prepared according to the procedure in Example 2, step 2 using 3,5-diiodo-4-hydroxybenzaldehyde (10.03 g, 26.83 mmol), 60% NaH/mineral oil (1.395 g, 34.87 mmol) and MEM chloride (4.902 mL, 42.92 mmol) to give 12.81 g, of the title compound. ^1H NMR (DMSO-d6) δ3.27 (s, 3 H), 3.54 (t, 2 H), 4.02 (t, 2 H), 5.24 (s, 2 H), 8.34 (s, 2 H), 9.84 (s, 1 H).

Step 2

2'-(2-Methoxyethoxymethoxy)-[1,1';3',1"]terphenyl-5'-carhaldehyde

The title compound was prepared according to the procedure in Example 2, step 3 using 3,5-diiodo-4-(2-methoxyethoxymethoxy)henzaldehyde (4.029 g, 8.721 mmol), phenylboronic acid (2.339 g, 19.19 mmol), palladium(II) acetate (39.16 mg, 0.174 mmol) in DME:H_2O. Purification on Biotage KP-Sil eluting with 15% EtOAc/pet. ether gave 1.803 g, (57%) of the title compound. ^1H NMR (DMSO-d6) δ2.78–2.82 (m, 2 H), 2.89–2.92 (m, 2 H), 3.01 (s, 3 H), 4,42 (s, 2 H), 7.40–7.54 (m, 6 H), 7.62×7.65 (m, 4 H), 7.91 (s, 2 H), 10.06 (s, 1 H).

Step 3

2'-(2-Methoxyethoxymethyl)-[1,1 ';3',1"]terphenyl-5'-carhoxylic acid

At ambient temperature, to a stirred mixture of silver (I) oxide (0.570 g, 2.46 mmol) in H_2O (9.8 mL) was added NaOH (0.983 g, 24.6 mmol) and the reaction was heated at

50° C. After 0.5 h, to the reaction was added a solution of 2'-(2-methoxyethoxymethoxy)-[1,1';3',1"]terphenyl-5'-carbaldehyde (1.78 g, 4.92 mmol) in THF (9.8 mL) and the reaction was heated at 90° C. for 1 h. To the reaction was added silver (I) oxide (0.570 g, 2.46 mmol), NaOH (0.983 g, 24.6 mmol) and H_2O(10.0 ML). The reaction was heated at 90° C. for 16 h. The reaction was filtered through a celite pad, rinsing with hot H_2O(3×). The filtrate was washed with ether (3×), acidified with 2N HCl (pH 2) and extracted with EtOAc. The organic extracts were dried ($MgSO_4$) and concentrated to give 1.73 g (93%) of the title compound. ^1H NMR (DMSO-d6) δ2.77–2.80 (m, 2 H), 2.88–2.91 (m, 2 H), 3.01 (s, 3 H), 4.39 (s, 2 H), 7.39–7.52 (m, 6 H), 7.59–7.62 (m, 4 H), 7.89 (s, 2 H), 13.07 (s, 1 H).

Step 4

2'-(2-Methoxyethoxymethyl)-[1,1';3',1"]terphenyl-5'-carboxylic acid methyl ester

At 0° C., to a stirred solution of 2'-(2-methoxyethoxymethoxy)-[1,1';3',1"]terphenyl-5'-carboxylic acid (1.72 g, 4.55 mmol) in THF (4.55 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene. After 0.5 h, to the reaction was added iodomethane and the reaction was stirred for 16 h, eventually warming to ambient temperature. The reaction was filtered and the filtrate concentrated. The crude product was dissolved in EtOAc, washed sequentially with sat. aq. $NaHCO_3$ (3×), with 1 N HCl (3×), brine (3×), dried ($K2CO_3$) and concentrated. Purification on Biotage KP-Sil eluting with 20% acetonelhexane gave 1.641 g (92%) of the title compound as a yellow oil. ^1H NMR (DMSO-d6) δ2.77–2.80 (m, 2 H), 2.88–2.91 (m, 2 H), 3.01 (s, 3 H), 3.87 (s, 3 H), 4.40 (s, 2 H), 7.40–7.53 (m, 6 H), 7.60–7.62 (m, 4 H), 7.90 (s,2 H).

Step 5

2'-Hydroxy-[1,1';3',1"]terphenyl-5'-carboxylic acid methyl ester

At ambient temperature, to a stirred solution of 2'-(2-methoxyethoxymethoxy)-[1,1';3',1"]terphenyl-5'-carboxylic acid methyl ester (1.608 g, 4.098 mmol) in CH_2Cl_2 (16.08 mL) was added portionwise trifluoroacetic acid (16.08 mL). After 1 h, the reaction was diluted with ether (100 mL), washed sequentially with H_2O (3×), with sat. aq. $NaHCO_3$ (3×), with brine (3×), dried ($MgSO_4$) and concentrated to give 1.240 g (99%) of the title compound as a white solid. ^1H NMR (DMSO-d6) δ3.83 (s, 3 H), 7.39–7.50 (m, 6 H), 7.55–7.57 (m, 4 H), 7.78 (s, 2 H), 9.28 (s, 1 H).

Step 6

2'-Methoxy-[1,1';3',1"]terphenyl-5'-carboxylic acid methyl ester

At ambient temperature, to a stirred mixture containing 2'-hydroxy-[1,1';3',1"]terphenyl-5'-carboxylic acid methyl ester (1.232 g, 4.049 mmol) and K_2CO_3 (0.6155 g, 4.454 mmol) in N,N-DMF (10.1 mL) was added iodomethane (0.353 mL, 5.67 mmol). After 18 h, the reaction was diluted with H_2O (100 mL) and the reaction was stirred vigorously. The resulting precipitate was collected, dissolved in CH_2Cl_2, dried (K_2CO_3) and concentrated to give 1.167 g, (99%) of the title compound. ^1H NMR (DMSO-d6) δ3.16 (s, 3 H), 3.87 (s, 3 H), 7.41–7.53 (m, 6 H), 7.58–7.60 (m, 4H) 7.89 (s, 2 H).

Step 7

2'-Methoxy-[1,1'; 3',1"]terphenyl-5'-carboxylic acid

At ambient temperature, to a stirred solution of 2'-methoxy-[1,1';3',1"]terphenyl-5'-carboxylic acid methyl ester (1.148 g, 3.606 mmol) in THF (36.06 mL) was added 1N KOH (36.06 mL) and the reaction was stirred for 16 h. The reaction was heated at 100° C. for an additional 24 h. The reaction was cooled to ambient temperature, diluted with ether (100 mL) and extracted with 0.1N NaOH. The aqueous extracts were washed with ether (3×), acidified with 6N HCl and extracted with EtOAc. The organic extracts were dried ($MgSO_4$) and concentrated to give 1.043 g (95%) of the tide compound. ^1H NMR (DMSO-d6) δ3.15 (s, 3 H), 7.40–7.52 (m, 6 H), 7.58–7.60 (m, 4 H), 7.88 (s, 2 H), 13.04 (s, 1 H).

Step 8

(2-Ethyl-benzofuran-3-yl)-(2'-methoxy-[1,1';3',1"]terphenyl-5'-yl)-methanone

The title compound was prepared according to the procedure in Example 5, step 2 using 2'-methoxy-[1,1';3',1"]terphenyl-5'-carboxylic acid (1.031 g, 3.388 mmol), oxalyl chloride (0.325 mL, 3.727 mmol), commercial 2-ethylbenzofuran (0.4953 g, 3.388 mmol) and tin (IV) chloride (0.436 mL, 3.727 mmol) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with 3% EtOAct pet. ether gave 1.070 g, (73%) of the title compound. ^1H NMR (DMSO-d6) δ1.24 (t, 3 H), 2.90 (quartet, 2 H), 3.21 (s, 3 H), 7.30–7.67 (m, 14 H), 7.75 (s, 2 H).

Step 9

(2-Ethyl-benzofuran-3-yl)-(2'-hydroxy-[1';3',1"]triphenyl-5'-yl)-methanone

At −78° C., to a stirred solution of (2-ethyl-benzofuran-3-yl)-(2'-methoxy-[1,1';3',1"]terphenyl-5'-yl)-methanone (1.07 g, 2.47 mmol) in CH_2Cl_2 (10.1 mL) was added dropwise 1M boron tribromidel CH_2Cl_2 (5.2 mL). After the addition was complete, the dry ice/acetone bath was replaced with an ice water bath and the reaction was stirred for 72 h, eventually warming to ambient temperature. The reaction was carefully quenched with crushed ice, diluted with H2O (16 mL) and extracted with ether. The ethereal extracts were dried ($MgSO_4$) and concentrated. Purification on Biotage KP-Sil eluting with 15% acetone/hexane gave 0.86 g, (83%) of the title compound as a white solid, mp 142–143° C. ^1H NMR (DMSO-d6)δ1.24 (t, 3 H), 2.90 (quartet, 2 H), 7.28–7.66 (m, 16 H), 9.37 (s, 1 H). IR (KBr) 3200, 2950, 1625, 1560 and 1175 cm^{-2}. mass spectrum (EI), m/z 418 (M+). Anal. Calcd. for $C_{29}H_{22}O_3$. 0.35H_2O: C, 82.00; H, 5.39; N, 0.00. Found: C, 82.06; H, 5.40; N, 0.07.

Step 10

4-[5'-(2-Ethyl-benzofuran-3-carbonyl)-[1,1';3',1"]triphenyl-2'-yloxysulfonyl]-2-hydroxy-benzoic acid sodium salt

The free acid of the title compound was prepared according to the procedure in Example 4 using (2-ethyl-benzofuran-3-yl)-(2'-hydroxy-[1,1';3',1"]terphenyl-5'-yl)-methanone (0.307 g, 0.734 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.208 g, 0.880 mmol). Purification on Dynamax C18 (85% CH_3CN/H_2O) followed by crystallization (EtOAc/hexane) gave 0.204 g, (45%) of the free acid as a white solid.

At ambient temperature, to a stirred solution of the free acid (0.204 g, 0.329 mmol) in MeOH (10 mL) was added 25% wt. sodium methoxidelmethanol (71.1 mg, 0.329 mmol). After 0.5 h, the reaction was concentrated to give 0.210 g (100%) of the title compound as a yellow solid, mp-211–212° C. ^1H NMR (DMSO-d6) δ 1.25 (t, 3 H), 2.45 (quartet, 2 H), 6.24–6.26 (m, 2 H), 7.28–7.33 (m, 6 H), 7.35–7.47 (m, 8 H), 7.65–7.67 (m, 1 H), 7.73 (s, 2 H). mass spectrum (−ESI), m/z 617 (M−H). Anal. Calcd. for $C_{36}H_{23}O_7SNa$. 1.H_2O: C, 65.11; H, 4.19; N, 0.00. Found: C, 64.96; H, 4.23; N, 0.10.

EXAMPLE 7

4-[5-(2-Benzyl4,5-dimethyl-furan-3-carbonyl)-3-methyl-biphenyl-2-yloxysulfonyl]-2-hydroxy-benzoic acid sodium salt

Step 1

3-Iodo-4-hydroxy-5-methylbenzaldehyde

At 5° C., to a stirred solution containing commercial 4-hydroxy-3-methylbenzaldehyde (4.65 g, 34.1 mmol) and sodium hydroxide (2.73 g, 68.25 mmol) in MeOH (171 mL)

was added iodine (10.8 g, 42.7 mmol). The reaction was stirred for 16 h, eventually warming to ambient temperature. The reaction was quenched with 1N HCl (200 mL) and extracted with ether. The ethereal extracts were washed with 10% $Na_2S_2O_3$ (3×), with brine (3×), dried ($MgSO_4$) and concentrated. Purification on Biotage KP-Sil eluting with 70% CH_2Cl_2/pet. ether to give 7.28 g (81%) of the tide compound as a yellow solid. ^1H NMR (DMSO-d6) δ2.30 (s, 3 H), 7.65 (d, J=1.02 Hz, 1 H), 8.09 (d, J=1.91 Hz, 1 H), 9.74 (s, 1 H), 10.25 (s, 1 H).

Step 2

3-Iodo-4-methoxy-5-methylbenzaldehyde

At 5° C., to a stirred solution of 3-iodo-4-hydroxy-5-methylbenzaldehyde (3.53 g, 13.5 mmol) in THF (67.4 mL) was added 60% sodium hydride/mineral oil (0.701 g, 17.5 mmol). After 0.5 h, to the reaction was added dropwise iodomethane (1.34 mL, 21.6 mmol). After 2 h, the reaction was heated at 60° C. After 3 days, the reaction was cooled to ambient temperature, quenched with H_2O (100 mL) and extracted with ether. The ethereal extracts were washed with 1N NaOH (3×), with brine (3×), dried (K_2CO_3) and concentrated. Purification on Biotage KP-Sil eluting with 15% acetone/hexane gave 2.24 g (60%) of the title compound. ^1H NMR (DMSO-d6) δ2.37 (s, 3 H), 3.77 (s, 3 H), 7.78 (s, 1 H), 8.17 (s, 1 H), 9.86 (s, 1 H).

Step 3

6-Methoxy-5-methyl-biphenyl-3-carbaldehyde

The tide compound was prepared according to the procedure in Example 2, step 3 using 3-iodo4-methoxy-5-methylbenzaldehyde (2.24 g, 8.11 mmol), phenylboronic acid (1.09 g, 8.92 mmol), palladium(II) acetate (36.4 mg, 0.162 mmol) and barium hydroxide octahydrate (3.83 g, 12.2 mmol) in DME-H_2O. Purification on Biotage KP-Sil eluting with 15% acetone/hexane gave 1.54 g (84%) of the title compound as a yellow oil. ^1H NMR (DMSO-d6) δ2.37 (s, 3 H), 3.39 (s, 3 H), 7.42–7.59 (m, 5 H), 7.74–7.79 (m, 2 H), 9.96 (s, 1 H).

Step 4

6-Methoxy-5-methyl-biphenyl-3-carboxylic acid

The tide compound was prepared according to the procedure in Example 6, step 3 using 6-methoxy-5-methyl-biphenyl-3-carbaldehyde (1.53 g, 6.77 mmol), silver (I) oxide (1.56 g, 6.77 mmol) and NaOH (2.71 g, 67.7 mmol) to give 1.46 g (89%) of the title compound as a white solid, mp 165–168° C. ^1H NMR (DMSO-d6) δ2.34 (s, 3 H), 3.36 (s, 3 H), 7.38–7.55 (m, 5 H), 7.71 (d, 1 H), 7.81 (dd, 1 H). IR (KBr) 3400, 2570, 1700, 1600 and 1410 cm^{-1}. mass spectrum (EI), m/z 242 (M+). Anal. Calcd. for $C_{15}H_{14}O_3$.0.2 H_2O: C, 73.27; H, 5.91; N, 0.00. Found: C, 73.21; H, 5.93; N, 0.17.

Step 5

(2-Benzyl-4,5-dimethyl-thiophen-3-yl)-(5-methyl-6-methoxy-biphen-3-yl)-methanone

The title compound was prepared according to the procedure in Example 5, step 2 using 6-methoxy-5-methyl-biphenyl-3-carboxylic acid (1.39 g, 5.74 mmol), 2-benzyl-4,5-dimethylfuran (1.15 g, 5.74 mmol), oxalyl chloride (0.550 mL, 6.32 mmol), tin (IV) chloride (0.739 mL, 6.32 mmol) and N,N-DMF (2 drops) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with 5% EtOAc/pet. ether gave 1.69 g (71%) of the tide compound as a brown oil. ^1H NMR (DMSO-d6) δ1.84 (s, 3 H), 2.18 (s, 3 H), 2.29 (s, 3 H), 3.38 (s, 3 H), 3.85 (s, 2 H), 7.04–7.07 (m, 2 H), 7.17–7.26 (m, 3 H), 7.38–7.48 (m, 6 H), 7.58 (d, 1 H).

Step 6

(2-Benzyl-4.5-dimethyl-thiophen-3-yl)-(5-methyl-6-hydroxy-biphen-3-yl)-methanone

The title compound was prepared according to the procedure in Example 5, step 3 using (2-benzyl-4,5-dimethyl-thiophen-3-yl)-(5-methyl-6-methoxy-biphen-3-yl)-methanone (1.68 g, 4.09 mmol) and 1M boron tribromide/CH_2Cl_2 (8.58 mL). Purification on Biotage KP-Sil eluting with 15% acetone/hexane gave 1.29 g (80%) of the title compound. ^1H NMR (DMSO-d6) δ1.83 (s, 3 H), 2.17 (s, 3 H), 2.25 (s, 3 H), 3.85 (s, 2 H), 7.07–7.10 (m, 2 H), 7.14–7.26 (m, 3 H), 7.33–7.43 (m, 6 H), 7.54 (d, 1 H), 9.31 (s, 1 H).

Step 7

4-[5-(2-Benzyl4,5-dimethyl-furan-3-carbonyl)-3-methyl-biphenyl-2-yloxysulfon]-2-hydroxy-benzoic acid sodium salt

The free acid of the tide compound was prepared according to the procedure in Example 4 using (2-benzyl-4,5-dimethyl-thiophen-3-yl)-(5-methyl-6-hydroxy-biphen-3-yl)-methanone (0.302 g, 0.762 =mol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.216 g, 0.991 mmol). Purification on Dynamax C18 (80% $CH_3CNlH20$) gave 0.15 g of the free acid.

The sodium salt was prepared according to the procedure in Example 6, step 10 using the free acid (0.15 g, 0.251 mmol) and 25% wt. sodium methoxide (54 mg, 0.251 mmol) in MeOH (7.6 mL) to give the tite compound as a yellow solid, mp 187–190° C. ^2H NMR (DMSO-d6) δ1.84 (s, 3 H), 2.18 (s, 3 H), 2.23 (s, 3 H), 3.83 (s, 2 H), 6.52 –6.56 (m, 2 H), 7.01–7.04 (m, 2 H), 7.14–7.24 (m, 8 H), 7.38 (d, 1 H), 7.56–7.60 (m, 2 H). IR (KBr) 3400, 2950, 1640, 1590 and 1430 cm^{-1}. mass spectrum (–LSI) m/z 595 (M–H). Anal. Calcd. for $C_{36}H_{27}O_8SNa$ .1.6 H_2O: C, 63.07; H, 4.70; N, 0.00. Found: C, 63.06; H, 4.60; N, 0.04.

EXAMPLE 8

4-[4-(4,5-Dimethyl-2-naphthalen-2-ylmethyl-furan-3-carbonyl)-2,6-diisopropyl-phenoxysulfonyl]-2-hydroxy-benzoic acid

Step 1

2-Naphthalen-2-ylmethyl-4,5-dimethylfuran

The title compound was prepared according to the procedure in Example 5, step 1 using 2,3-dimethylfuran, 2-(bromomethyl)naphthalene and 1.6M n-BuLi/hexanes in THF. ^1H NMR (DMSO-d6) δ1.89 (s, 3 H), 2.15 (s, 3 H), 4.08 (s, 2 H), 5.95 (s, 1 H), 7.40 (d, 1 H), 7.45–7.57 (m, 2 H), 7.77 (s, 1 H), 7.83–7.91 (m, 3 H).

Step 2

(2-Naphthalen-2-ylmethyl–4,5-dimethyl-furan-3-yl)-(3,5-diisopropyl-4-methoxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 2 using 3,5-diisopropyl-p-anisic acid (0.93 g, 3.95 mmol, RN-117439-59-5), oxalyl chloride (0.377 mL, 4.33 mmol), N,N-dimethylformamide (2 drops), tin (IV) chloride (0.507 mL, 4.33 mmol) and 2-naphthalen-2-ylmethyl4,5-dimethylfuiran (0.93 g, 3.94 mmol) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with a 2 & 4% EtOAc/pet. ether step gradient gave 0.357 g (20%) of the title compound. ^1H NMR (DMSO-d6) δ1.14 (d, 12 H), 1.82 (s, 3 H), 2.20 (s, 3 H), 3.27 (septet, 2 H), 3.74 (s, 3 H), 4.02 (s, 2 H), 7.19 (dd, 1 H), 7.45–7.52 (m, 5 H), 7.76–7.79 (m, 3 H).

Step 3

(2-Naphthalen-2-ylmethyl-4.5-dimethyl-furan-3-yl)-(3,5-diisopropyl-4-hydroxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 3 using (2-naphthalen-2-ylmethyl-4,5-dimethyl-furan-3-yl)-(3,5-diisopropyl-4-methoxy-phenyl)-methanone (1.04 g, 2.29 mmol) and 1M boron tribroride/CH_2Cl_2 (4.81 mL) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with 15% acetone/hexane gave 0.542 g (54%) of the title compound. ^1H NMR (DMSO-d6)

δ1.12 (d, 12 H), 1.82 (s, 3 H), 2.18 (s, 3 H), 3.31 (septet, 2 H), 4.02 (s, 2 H), 7.22 (dd, 1 H), 7.43–7.50 (m, 4 H), 7.55 (s, 1 H), 7.76–7.86 (m, 3 H), 9.12 (s, 1 H).

Step 4

4-14-(4,5-Dimethyl-2-nalthtalen-2-ylmethyl-furan-3-carhonyl)-2,6-diisopropyl-phenoxysulfonyl]-2-hydroxy-henzoic acid

The title compound was prepared according to the procedure in Example 4 using (2-naphthalen-2-ylmethyl-4,5-dimethyl-furan-3-yl)-(3,5-diisopropyl-4-hydroxy-phenyl)-methanone (0.300 g, 0.681 mmol) and 4-chlorosulphonyl-2-hydroxybonzoic acid (0.209 g, 0.885 mmol). Purification on Dynamax C18 (80% CH_3CN/H_2O) gave 0.240 g (55%) of the title compound as a yellow solid, mp 130–135° C. ^1H NMR (DMSO-d6) δ1.03 (d, 12 H), 1.83 (s, 3 H), 2.20 (s, 3 H), 3.09 (septet, 2 H), 4.01 (s, 2 H), 7.18 (d, 1 H), 7.43–7.48 (m, 5 H), 7.51 (s, 2 H), 7.77–7.79 (m, 2 H). 7.82–7.86 (m, 1 H), 8.05 (d, 1 H). IR (KBr) 3400, 2950, 1700, 1375 and 1190 cm^{-1}. mass spectrum –(ESI) m/z 639 (M–H). Anal. Caled. for $C_{37}H_{36}O_8S$: C, 69.36, H, 5.66; N, 0.00. Found: C, 69.04; H, 5.77; N, 0.04.

EXAMPLE 9

4-[4-[2-(2-Bromo-benzyl)-4.5-dimethyl-furan-3-carbonyl]-2,6-diisopropyl-phenoxysulfonyl}-2-hydroxy-henzoic acid

Step 1

2-(2-Bromo-benzyl)-4.5-dimethyl-furan

The title compound was prepared according to the procedure in Example 5, step 1 using commercial 2,3-dimethylfuran (1.00 g, 10.4 mmol), commercial 2-bromobenzylhromide (2.60 g, 10.4 mmol) and 1.6M n-BuLi/hexanes (4.16 mL, 10.4 mmol) in THF. Purification on Biotage KP-Sil eluting with 100% pet. ether gave 1.14 g (41%) of the title compound as a clear oil. ^1H NMR (DMSO-d6) δ1.84 (s, 3 H), 2.11 (s, 3 H), 3.96 (s, 2 H), 5.81 (s, 1 H), 7.19 (dt, 1 H), 7.26–7.37 (m, 2 H), 7.60 (dd, 1 H).

Step 2

[2-(2-Bromo-benzyl)-4.5-dimethyl-furan-3-yl]-(3,5-diisopropyl-4-methoxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 2 using 3,5-diisopropyl-p-anisic acid (0.983 g, 4.16 mmol, RN-117439-59-5), oxalyl chloride (0.398 mL, 4.56 mmol), N,N-dimethylformamide (2 drops), tin (IV) chloride (0.535 mL, 4.58 mmol) and 2-(2-bromobenzyl)4,5-dimethyl-furan (1.10 g, 4.16 mmol) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with 3% EtOAc/pet. ether gave 0.990 g (49%) of the title compound as a clear oil. ^1H NMR (DMSO-d6) δ1.15 (d, 12 H), 1.84 (s, 3 H), 2.18 (s, 3 H), 3.27 (septet, 2 H), 3.73 (s, 3 H), 3.91 (s, 2 H), 7.12–7.16 (m, 2 H), 7.29 (t, 1 H, 7.44 (s, 2 H), 7.50 (d, 1 H).

Step 3

2-(2-Bromo-benzyl)4,5-dimethyl-furan-3-yl]-(3.5-diisopropyl-4-hydroxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 3 using [2-(2-bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3,5-diisopropyl-4-methoxy-phenyl)-methanone (0.973 g, 2.01 mmol) and 1M boron tribromide/CH_2Cl_2 (4.23 mL) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with 15% acetone/hexane gave 0.679 g (72%) of the title compound. ^1H NMR (DMSO-d6) δ 1.13 (d, 12 H), 1.82 (s, 3 H), 2.18 (s, 3 H), 3.31 (septet, 2 H), 3.94 (s, 2 H), 7.12–7.17 (m, 2 H), 7.30 (di, 1 H), 7.42 (s, 2 H), 7.52 (d, 1 H), 9.10 (s, 1 H).

Step 4

4-[4-[2-(2-Bromo-henzyl)-4,5-dimethyl-furan-3-carhonyl]-2,6-diisopropyl-phenylsulfonyl}-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 4 using [2-(2-bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3,5-diisopropyl-4-hydroxy-phenyl)-methanone (0.300 g, 0.639 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.197g, 0.831 mmol). Purification on Dynamax C18 (90% CH_3CN/H_2O) gave 0.20 g (47%) of the tide compound as a yellow solid, mp 90–95° C. ^1H NMR (DMSO-d6) δ1.06 (d, 12 H), 1.85 (s, 3 H), 2.19 (s, 3 H), 3.07 (septet, 2 H), 3.91 (s, 2 H), 7.14–7.16 (m, 2 H), 7.29 (dt, 1 H), 7.44 (d, 1 H), 7.47–7.52 (m, 4 H), 8.05 (d, 1 H) (KBr) 3400, 2950, 1690, 1380 and 1190 cm^{-1}. mass spectrum –(EST) mz 667/669 (M–H). Anal. Caled. for $C_{34}H_{38}BrO_8S$: C, 59.19; H, 4.97; N, 0.00. Found: C, 58.82; H, 5.13; N, 0.13.

Example 10

4-[4-[2-(3-Bromo-henzyl)-4.5-dimethyl-furan-3-carbonyl]-2,6-diisopropylphenoxysulfonyl]-2-hydroxy-benzoic acid

Step 1

2-(3-Bromo-benzyl)4,5-dimethyl-furan

The title compound was prepared according to the procedure in Example 5, step 1 using commercial 2,3-dimethylfuran (5.00 g, 52.0 mmol), commercial 3-bromobenzylbromide (13.0 g, 52.0 mmol) and 2.5M n-BuLi/hexanes (20.8 mL, 52.0 mmol) in THF. Purification on Biotage KP-Sil eluting with 1% EtOAc/pet. ether gave 12.63 g (85%) of the title compound. ^1H NMR (DMSO-d6) δ1.85 (s, 3 H), 2.10 (s, 3 H), 3.86 (s, 2 H), 5.90 (s, .1 H), 7.21–7.30 (m, 2 H), 7.34–7.47 (m, 2 H).

Step 2

[2-(3-Bromo-benzyl)-4.5-dimethyl-furan-3-yl]-(3.5-diisopropyl-4-methoxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 2 using 3,5-diisopropyl-p-anisic acid (1.02 g, 4.32 mmol, RN-1 17439-59-5), oxalyl chloride (0.415 mL, 4.75 mmol), N,N-dimethylformamide (2 drops), tin (IV) chloride (0.556 mL, 4.75 mmol) and 2-(3-bromobenzyl)-4,5-dimethyl-furan (1.15 g, 4.32 mmol) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with 3% EtOAc/pet ether gave 1.36 g (65%) of the title compound as a yellow clear oil. ^1H NMR (DMSO-d6) δ1.17 (d, 12 H), 1.80 (s, 3 H), 2.20 (s, 3 H), 3.28 (septet, 2 H), 3.74 (s, 3 H), 3.87 (s,2 H), 7.06 (d, 1 H), 7.19 (d, 1 H), 7.24 (s, 1 H), 7.38 (dd, 1 H), 7.46 (s, 2 H).

Step 3

[2-(3-Bromo-henzyl)-4.5-dimethyl-furan-3-yl]-(3.5-diisopropyl-4-hydroxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 3 using [2-(3-bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3,5-diisopropyl-4-methoxy-phenyl)-methanone (1.34 g, 2.78 mmol) and 1M boron tribromide/CH_2Cl_2 (5.84 mL) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with 15% acetonelhexane gave 0.995 g (73%) of the title compound. ^1H NMR (DMSO-d6) δ1.14 (d, 12 H), 1.80 (s, 3 H), 2.19 (s, 3 H), 3.32 (septet, 2 H), 3.87 (s, 2 H), 7.09 (d, 1 H), 7.22 (t, 1 H), 7.26 (s, 1H), 7.39 (d, 1 H), 7.43 (s, 2 H), 9.13 (s, 1 H). IR (KBr) 3350, 2950, 1580, 1560 and 1325 cm^{-1}. mass spectrum (EI) m/z 468 (M+). Anal. Caled. for $C_{25}H_{29}BrO_3$: C, 66.53; H, 6.23; N, 0.00. Found: C, 66.50; H, 6.22; N, 0.08.

Step 4

4-[4-[2-(3-Bromo-henzyl)-4,5-dimethyl-furan-3-carhonyl]-2.6-diisopropyl-phenoxysulfonyl}-2-hydroxy-benzoic acid

The tide compound was prepared according to the procedure in Example 4 using [2-(3-bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3,5-diisopropyl-4-hydroxy-phenyl)-methanone (0.300 g, 0.639 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.197 g, 0.831 mmol). Purification

on Dynamax C18 (95% CH_3CN/H_2O) gave 0.23 g (54%) of the title compound as an off white solid, mp 148° C. ^1H NMR (DMSO-d6) δ1.06 (d, 12 H), 1.80 (s, 3 H), 2.21 (s, 3 H), 3.08 (septet, 2 H), 3.87 (s, 2 H), 1 H), 7.21 (t, 1 H), 7.25 (s, 1 H), 7.38 (d, 1 H), 7.44 (s, 1 H), 7.48 (dd, 1 H), 7.51 (s, 2 H), 8.06 (d, 1 H). IR (KBr) 3400, 2950, 1680, 1650 and 1180 cm^{-1}. mass spectrum (−ESI) m/z 667 (M−H). Anal. Calcd. for $C_{33}H_{33}BrO_8S$: C, 59.19; H, 4.97; N, 0.00. Found: C, 58.86; H, 4.93; N, 0.07.

EXAMPLE 11

4-{4-[2-(4-Bromo-benzyl)4,5-dimethyl-furan-3-carbonyl]-2,6-diisopropyl-phenoxysulfonyl}-2-hydroxy-benzoic acid

Step 1

2-(4-Bromo-benzyl)4,5-dimethyl-furan

The title compound was prepared according to the procedure in Example 5, step 1 using commercial 2,3-dimethylfuran (5.00 g, 52.0 mmol), commercial 4-bromobenzylbromide (13.0 g,52.0 mmol) and 2.5M n-BuLi/hexanes (20.8 mL, 52.0 mmol) in THF. Purification on Biotage KP-Sil eluting with 1% EtOAc/pet. ether gave 5.72 g (41%) of the title compound. ^1H NMR (DMSO-d6) δ1.84 (s, 3 H), 2.09 (s, 3 H), 3.83 (s, 2 H), 5.87 (s, 1 H), 7.17 (d, 2 H), 7.49 (d, 2 H).

Step 2

[2(4-Bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3,5-diisopropyl-4-methoxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 2 using 3,5-diisopropyl-p-anisic acid (1.03 g, 4.35 mmol, RN-117439-59-5), oxalyl chloride (0.417 mL, 4.79 mmol), N,N-dimethylformamide (2 drops), tin (TV chloride (0.560 mL, 4.79 mmol) and 2-(4-bromo-benzyl)4,5-dimethyl-furan (1.15 g, 4.35 mmol) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with 3% EtOAc/pet. ether gave 1.63 g (77%) of the title compound as a yellow clear oil. ^1H NM (DMSO6) δ1.16 (d, 12 H), 1.80 (s, 3 H), 2.19 (s, 3 H), 3.28 (septet, 2 H), 3.74 (s, 3 H), 3.83(s, 2 H), 6.98 (d, 2 H), 7.41 (dd, 2 H), 7.44 (s, 2 H).

Step 3

[2-(4-Bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3,5-diisopropl)-4-hydroxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 3 using [2-(4-bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3,5-diisopropyl-4-methoxy-phenyl)-methanone (1.61 g, 3.32 mmol) and 1M boron tribromide/CH_2Cl_2 (6.97 mL) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with 15% acetone/hexane gave 1.37 g (88%) of the title compound. ^1H NMR (DMSO-d6) δ1.12 (d, 12 H), 1.78 (s, 3 H), 2.17 (s, 3 H), 3.30 (septet, 2 H), 3.81 (s, 2 H), 7.01 (dd, 2 H), 7.40 (s, 2 H), 7.42 (d, 2 H), 9.10 (s, 1 H). IR (KBr) 3400, 2950, 1640, 1580 and 1310 cm^{-1}. mass spectrum (EI) m/z 468 (M+). Anal. Calcd. for $C_{26}H_{29}BrO_3$: C, 66.53; H, 6.23; N, 0.00. Found: C, 66.57; H, 6.39; N, 0.05.

Step 4

4-{4-[2-(4-Bromo-benzyl)-4.5-dimethyl-furan-3-carbonyl]-2,6-diisopropyl-phenoxysulfonyl}-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 4 using [2-(4-bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3,5-diisopropyl-4-hydroxy-phenyl)-methanone (1.30 g, 2.77 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (1.00 g, 4.22 mmol). Purification on Dynamax C18 (90% CH_3CN/H_2O) gave 0.98 g (53%) of the title compound as an off white solid, mp 165–168° C. ^1H NMR (DMSO-d6) δ1.05 (d, 12 H), 1.80 (s, 3 H), 2.19 (s, 3 H), 3.06 (septet, 2 H), 3.81 (s, 2 H), 6.92 (d 2 H), 7.39 (dd, 2 H), 7.44 (s, 1 H), 7.45 (s, 2 H), 7.48 (d, 1 H), 8.05 (d, 1 H). IR (KBr) 3400, 2950, 1680, 1375 and 1190 cm^{-1}. mass spectrum (−ESI) m/z 667 (M−H). Anal. Calcd. for $C_{33}H_{33}BrO_8S$: C, 59.20; H, 4.97; N, 0.00. Found: C, 59.18; H, 5.21; N, 0.08.

EXAMPLE 12

4-{4-[2-(2-Benzyl-benzo[b]thiophen-3-yl)thyl]-phenoxysulfonyl }-2-hydroxy-benzoic acid

Step 1

2-Benzyl-benzo[b]thiophene-3-carboxaldehyde

Tin chloride (14.0 mL, 119.6 mmol) was added dropwise into a cold (−20 ° C.) solution of 2-benzyl-benzo[b] thiophene (13.4 g, 59.8 mmol) in dichloromethane (150.0 mL). After 1 h, to the reaction was added dropwise dichloromethyl methyl ether (119.6 mmol). The mixture was allowed to gradually come to 0 ° C., and then stirred for 20 hours. The mixture was carefully poured into HCl (2 N) and ice, and extracted with ethyl ether. The ethereal extracts were dried ($MgSO_4$) and concentrated to give 12.9 g (85%) of the title compound after crystallization from ethyl ether/hexanes, yellow solid mp 86–88 ° C.; mass spectrum m/z 252 (M+). Anal. Calcd. for $C_{16}H_{12}O_2$: C, 76.16; H, 4.79. Found: C, 75.77; H, 4.85

Step 2

trans-2-Benzyl-3-[2-(4-methoxy-phenyl)-vinyl]-benzo[b] thiophene & cis-2-Benzyl-3-[2-(4-methoxy-phenyl)-vinyl]-benzo[b]thiophene

At −78° C., to a stirred suspension of (4-methoxybenzyl) triphenylphosphonium chloride (0.996 g, 2.38 mmol) in THF was added dropwise 1.6M n-BuLi/hexanes (1.36 mL, 2.18 mmol). After 1 h, to the reaction was added dropwise to a solution of 2-benzyl-benzo[b]thiophene-3-carboxaldehyde (5.00 g, 26.8 mmol) in THF that had been previously cooled to −78° C. The reaction was allowed to warm to ambient temperature and stirred for 2 h. The reaction was quenched with H_2O (200 mL), extracted with CH_2Cl_2 and concentrated. Purification on Biotage KP-Sil eluting with a 1% EtOAc/pet ether gave the title compounds as white solids. Analytical data trans isomer; mp 79–81° C. ^1H NMR (DMSO-d6) δ3.66 (s, 3 H), 4.01 (s, 2 H), 6.55 (d, 1 H), 6.71 (d, 2 H), 6.88 (d, 1 H), 7.01 (d, 2 H), 7.13–7.29 (m, 7 H), 7.39–7.42 (m, 1 H), 7.83–7.85 (m, 1 H). IR (KBr) 3100, 2950, 1600, 1510 and 1250 cm^{-1}. mass spectrum (EI) m/z 356 (M+). Anal. Calcd. for $C_{24}H_{20}OS$ .0.2H_2O: C, 80.05; H, 5.71; N, 0.00. Found: C, 80.22; H, 5.86; N, 0.04. Analytical data cis isomer; mp 95–98° C. ^1HNMR (DMSO-d6) δ3.78 (s, 3 H), 4.41 (s, 2 H), 6.95 (d, 2 H), 7.07 (d, 1 H), 7.21–7.24 (m, 1 H), 7.29–7.43 (m, 7 H), 7.61 (d, 2 H), 7.87 (dd, 1 H), 8.06 (d, 1 H). IR (KBr) 3050, 2900, 1700, 1500 and 1250 cm^{-1}. mass spectrum (EI) m/z 356 (M +). Anal. Calcd. for $C_{24}H_{20}OS.0.1H_2O$: C, 80.46; H, 5.68; N, 0.00. Found: C, 80.40; H, 5.95; N, 0.01.

Step 3

2-Benzyl-3-[2-(4-methoxy-phenyl)-ethyl]-benzo[b] thiophene

At ambient temperature, a mixture of cis and trans-2-benzyl-3-[2-(4-methoxy-phenyl)-viny]-benzo[b]thiophene in EtOAc and 10% palladium on carbon was stirred under H_2 (atmospheric conditions). The reaction was filtered and the filtrate concentrated to give the title compound as a white solid, mp 68–70° C. ^1H NMR; consistent. mass spectrum (EI) m/z 358 (M+). Anal. Calcd. for $C_{24}H_{22}OS$: C, 80.41; H, 6.19; N, 0.00. Found: C, 80.20; H, 6.18; N, 0.03.

Step 4

4-[2-(2-Benzyl-benzo[b]thiophen-3-yl)-ethyl]-phenol

The title compound was prepared according to the procedure in Example 5, step 3 using 2-benzyl-3-[2-(4-

methoxy-phenyl)-ethyl]-benzo[b]thiophene (5.50 g, 15.3 mmol) and 1M boron tribromide/CH_2Cl_2 (49.1 mL) in CH_2Cl_2. Purification on silica gel eluting with a 5 & 10% EtOAc/pet. ether step gradient gave the title compound as an oil. ^1H NMR; consistent. mass spectrum (EI) m/z 344 (M+).

Step 5

4-{4-[2-(2-Benzy]-benzo[b]thiophen-3-yl)-ethyl]-phenoxysulfonyl}-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 4 using 4-[2-(2-benzyl-benzo[b] thiophen-3-yl)-ethyl]-phenol (0.400 g, 1.16 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.550 g, 2.32 mmol). Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with 20% EtOAc/hexane gave the title compound as an off white solid, mp 57–59° C. ^1H NMR (DMSO-d6) 82.74 (t, 2 H), 3.09 (t, 2 H), 4.00 (s, 2 H), 6.97 (d, 2 H), 7.19–7.23 (m, 5 H), 7.27–7.38 (m, 6 H), 7.76 (d 1 H), 7.83 (dd, 1 H), 7.98 (d, 1 H). IR (KBr) 3400, 2950, 1675, 1390 and 1190 cm^{-1} . mass spectrum (–ESI) m/z 543 (M–H).

EXAMPLE 13

4-{4-[2-(4-Bromo-benzyl)4,5-dimethyl-furan-3-carbonyl]-2-cyclopentyl-phenylsulfonyl}-2-hydroxy-benzoic acid

Step 1

[2-(4-Bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3-cyclopentyl-4-methoxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 2 using 3-cyclopentyl-p-anisic acid (2.04 g, 9.24 mmol, RN 59216-82-9), oxalyl chloride (0.887 mL, 10.2 mmol), N,N-dimethylformamide (2 drops), tin (IV) chloride (1.19 mL, 10.2 mmol) and 2-(4-bromo-benzyl)-4,5-dimethyl-furan (2.45 g, 9.24 mmol) in $CH2C_2$. Purification on Biotage KP-Sil eluting with 4% EtOAc/pet. ether gave 3.39 g (78%) of the title compound. ^1H NMR (DMSO-d6) δ1.38–1.47 (m, 2 H), 1.60–1.73 (m, 4 H), 1.79 (s, 3 H), 1.90–1.94 (m, 2 H), 2.18 (s, 3 H), 3.21 (quintet, 1 H) 3.82 (s, 2 H), 3.88 (s, 3 H), 7.02–7.08 (m, 3 H), 7.44 (d, 2 H), 7.55–7.61 (m , 2 H).

Step 2

[2-(4-Bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3-cyclopentyl-4-hydroxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 3 using [2-(4-bromo-benzyl)4,5-dimethyl-faran-3-yl]-(3-cyclopentyl-4-methoxy-phenyl)-methanone (3.38 g, 7.23 mmol) and 1M boron tribromide/CH_2Cl_2 (15.2 mL) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with 15% acetone/hexane gave 1.66 g (51%) of the title compound. ^1H NMR (DMSO-d6) δ1.39–1.48 (m, 2 H), 1.59–1.65 (m, 2 H), 1.68–1.76 (m, 2 H), 1.79 (s, 3 H), 1.88–1.95 (m, 2 H), 2.18 (s, 3 H), 3.18 (quintet, 1 H), 3.81 (s, 2 H), 6.87 (d, 1 H), 7.04 (d, 2 H), 7.43–7.47 (m, 3 H), 7.53 (s, 1 H), 10.35 (s, 1 H). IR (KBr) 3300, 2950, 1650, 1575 and 1280 cm^{-1}. mass spectrum (+ESI) m/z 453 (M+H). Anal. Calcd. for $C_{25}H_{25}BrO_2$: C, 66.23; H, 5.56; N, 0.00 Found: C, 66.12; H, 5.49; N, 0.03.

Step 3

4-{4-[2-(4-Bromo-benzyl)4,5-dimethyl-furan-3-carbonyl]-2-cyclopentyl-phenoxysulfonyl}-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 4 using [2-(4-bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3-cyclopentyl4-hydroxy-phenyl)-methanone (0.203 g, 0.448 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.319 g,1.34 mmol). Purification on Dynamax C18 (85% CH_3CNH_2O) gave 0.221 g (49%) of the title compound as a brown solid, mp 188–192° C. ^1H NMR (DMSO-d6) δ1.26–1.32 (m, 2 H), 1.49–1.57 (m, 2 H), 1.64–1.74 (m, 4 H), 1.77 (s, 3 H), 2.18 (s, 3 H), 2.96 (quintet, 1 H), 3,79 (s, 2 H), 6.96 (d, 2 H), 7.23 (d, 1 H), 7.36 –7.43 (m, 4 H), 7.53–7.56 (m, 2 H), 8.00 (d, 1 H). IR (KBr) 3400, 2950, 1690, 1390 and 1190 cm^{-1}. mass spectrum (–ESI) m/z 651 (M–H). Anal. Calcd. for $C_{32}H_{29}BrO_6S$: C, 58.81; H, 4.47; N, 0.00. Found: C, 58.45; H, 4.54; N, 0.07.

EXAMPLE 14

4-{4-[2-(2-Butyl-benzofuran-3-yl)-ethyl]-phenoxysulfonyl}-2-hydroxy-benzoic acid

Step 1

2-n-Butylbenzofuran-3-carboxaldehyde

At ambient temperature, phosphorous oxychloride (180 mL, 1.94 mol) was carefully added dropwise to N,N-DMF (200 mL). After the addition was complete the reaction was cooled to 10° C. To the reaction was added dropwise commercial 2-butylbenzofuran (240 mL, 1.37 mol) and the reaction was heated to 85° C. for 70 h. The reaction was cooled to ambient temperature and carefully quenched with H_2O (600 mL), adjusted to pH 5 with NaOAc and extracted with ether. The ethereal extracts were washed with H_2O, dried ($MgSO_4$) and concentrated. The crude product was purified on silica gel eluting with a 0% & 20% CH_2Cl_2/hexane step gradient to give 186 g (84%) of the title compound as a yellow oil. ^1H NMR consistent.

Step 2

cis-2-Butyl-3-[2-(4-methoxu-phenyl)-vinyl]-benzofuran & trans-2-Butyl-3-[2-(4-methoxy-phenyl)-vinyl]-benzofuran

The title compound was prepared according to the procedure in Example 12, step 2 using (4-methoxybenzyl) triphenylphosphonium chloride (13.5 g, 32.2 mmol), 1.6M n-BuLi/hexanes (18.5 mL, 29.5 mmol) and 2-n-butylbenzofuran-3-carboxaldehyde (5.00 g, 26.8 mmol). Purification on Biotage KP-Sil eluting with a 10 & 20% toluene/pet. ether step gradient gave the title compounds as oils. Analytical data cis isomer: ^1H NMR consistent. IR (KBr) 2950, 1600, 1560, 1450 and 1250 cm^{-1}. mass spectrum (EI) m/z 306 (M+). Analytical data trans isomer: ^1H NMR consistent. mass spectrum (EI) m/z 306 (M+).

Step 3

2-Butyl-3-[2-(4-methoxy-phenyl)-ethyl]-benzofuran

At ambient temperature, a solution containing cis-2-butyl-3-[2-(4-methoxy-phenyl)-vinyl]-benzofuran (0.400 g, 1.31 mmol) in EtOAc (5 mL) and 10% palladium on carbon (200 mg) was stirred under H_2 (atmospheric conditions). The reaction was filtered and the filtrate concentrated to the title compound as a clear oil. ^1H NMR (DMSO-d6) δ0.83 (t, 3 H), 1.20 (sextet, 2 H), 1.37 (quintet, 2 H), 2.49–2.52 (m, 2 H, with DMSO), 2.78–2.85 (m, 4 H), 3.68 (s, 3 H), 6.78 (d, 2 H), 7.03 (d, 2 H), 7.17–7.21 (m, 2 H), 7.41–7.43 (m, 1 H), 7.54–7.56 (m, 1 H). IR (KBr) 2950, 1610, 1510, 1450 and 1250 cm^{-1}. mass spectrum (EI) m/z 308 (M +). Anal. Calcd. for $C_{21}H_{24}O_2$: C, 81.78; H, 7.84; N, 0.00. Found: C, 81.55; H, 7.57; N, 0.10.

Step 4

4-[2-(2-Butyl-benzofuran-3-yl)-ethyl]-phenol

The title compound was prepared according to the procedure in Example 5, step 3 using 2-butyl-3-[2-(4-methoxy-phenyl)-ethyl]-benzofuran (0.300 g, 0.973 mmol) and 1M boron tribromide/CH_2Cl_2(3.11 mL) in CH_2Cl_2. Purification on silica gel eluting with 50% EtOAc/pet. ether gave the title compound as a dark oil. ^1H NMR (DMSO-d6) δ0.84 (t, 3 H), 1.22 (sextet, 2 H), 1.41 (quintet, 2 H), 2.52 (t, 2 H), 2.74 (t, 2 H) 2.81 (t, 2 H), 6.61 (d, 2 H), 6.91 (d, 2 H), 7.15–7.21 (m, 2 H), 7.40–7.43 (m, 1 H), 7.52–7.54 (m, 1 H), 9.10 (s, 1 H). IR (KBr) 3400, 2950, 1610, 1510 and 1450 cm^{-1}. mass spectrum (FAB) m/z 295 (M+H).

Step 5

4-{4-[2-(2-Butyl-benzofuran-3-yl)-ethyl]-phenoxysulfony}-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 4 using 4-[2-(2-butyl-benzofuran-3-yl)-ethyl]-phenol (0.410 g, 1.36 mmol) and 4-chlorosulphonyl-

2-hydroxybenzoic acid (0.644 g, 2.72 mmol). Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with EtOAc/hexane gave 0.571 g (85%) of the title compound as a light brown solid, mp 93–95° C. ^1H NMR consistent. mass spectrum (–ESI) m/z 493 (M–H).

EXAMPLE 15

4-[4-[(2-Butyl-benzofuran-3-ylmethyl)-amino]-phenoxysulfonyl]-2-hydroxy-benzoic acid

Step 1

4[(2-Butyl-benzofuran-3-ylmethyl)-amino]-phenol

At ambient temperature, to a stirred solution containing 2-n-butylbenzofiran-3-carboxaldehyde (0.888 g, 4.77 mmol) and 4-aminophenol hydrochloride in MeOH (7 mL) was added sodium cyanoborohydride (0.330 g, 5.25 mmol). After 24 h, the reaction was carefully quenched with 3N HCl, diluted with H_2O and extracted with CH_2Cl_2. The organic extracts were washed with sat. aq. $NaHCO_3$, dried and concentrated. Purification on silica gel eluting with a 20 & 40% EtOAc/pet. ether step gradient gave the title compound as a pale yellow solid. ^1H NMR ($CDCl_3$) δ 0.93 (t, 3 H), 1.38 (sextet, 2 H), 1.70 (quintet, 2 H), 2.79 (t, 2 H), 4.27 (s, 1 H), 4.35 (br s, 1 H), 6.62 (d, 2 H), 6.75 (d, 2 H), 7.18–7.26 (m, 3 H), 7.41 (d, 1 H), 7.54 (d, 1 H).

Step 2

4-{4-[(2-Butyl-benzofuran-3-ylmethyl)-amino]-phenoxysnlfonyl}-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 4 using 4-[(2-butyl-benzofuran-3-ylmethyl)-amino]-phenol (0.500 g, 1.69 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.401 g, 1.69 mmol). Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with 25% EtOAc/hexane gave 0.571 g (85%) of the title compound as a dark yellow solid, mp 75–77° C. ^1H NMR (DMSO-d6) δ0.86 (t, 3 H), 1.31 (sextet, 2 H), 1.60 (quintet, 2 H), 2.80 (t, 2 H), 4.23 (s, 2 H), 6.54 (b, 2 H), 6.72 (d, 2 H), 7.14–7.22 (m, 2 H), 7.26–7.30 (m, 2 H), 7.45 (dd, 1 H), 7.58 (dd, 1 H), 7.95 (d, 1 H). IR (KBr) 3400, 2950, 1600, 1510 and 1380 cm^{-1}. mass spectrum (–ESI) m/z 494 (M–H).

EXAMPLE 16

4-[4-(2-Butyl-benzofuran-3-carbonyl)-phenylsulfonyl]-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 4 using commercial 2-n-butyl-3-(4-hydroxybenzoyl)benzofuran (1.00 g, 3.40 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (1.61 g, 6.79 mmol). Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with EtOAc/hexane gave 0.185 g (11%) of the title compound as an off white solid, mp 143–145° C. ^1H NMR (DMISO-d6) 80.76 (t, 3 H), 1.12–1.22 (m, 2 H), 1.60 (quintet, 2 H), 2.69 (t, 2 H), 7.21–7.38 (m, 7 H), 7.61 (d, 1 H), 7.76 (d, 2 H), 7.98 (d, 1 H). IR (KBr) 2950, 1690, 1600, 1380 and 1150 cm^{-1}. mass spectrum (+ESI) m/z 495 (M+H). Anal. Calcd. for $C_{26}H_{22}O_6S$. 0.2H_2O: C, 62.24; H, 4.58; N, 0.00. Found: C, 62.38; H, 4.59; N, 0.15.

EXAMPLE 17

4-[4-(2-Benzyl-4,5-dimethyl-furan-3-carbonyl)-2-cyclopentyl-phenoxysulfonyl]-2-hydroxy-benzoic acid

Step 1

(2-Benzyl-4,5-dimethyl-furan-3-yl)-(3-cyclopentyl-4-methoxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 2 using 3-cyclopentyl-p-anisic acid (10.0 g, 45.5 mmol, RN-59216-82-9), oxalyl chloride (4.4 mL, 50.6 mmol), N,N-dimethylformamide (2 drops), tin (IV) chloride (15.8 mL, 49.6 mmol) and 2-benzyl-4,5-dimethyl-furan (10.1 g, 54.3 mmol) in CH_2Cl_2 to give 18.8 g (crude) of the title compound. ^1H NMR (DMSO-d6) consistent.

Step 2

(2-Benzyl-4.5-dimethyl-furan-3-yl)-(3-cyclopentyl-4-hydroxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 3 using (2-benzyl-4,5-dimethyl-furan-3-yl)-(3-cyclopentyl4-methoxy-phenyl)-methanone (17.7 g, 45.5 mmol) and 1M boron tribromide/CH_2Cl_2 (34.8 mL) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with a 2, 5 & 10% EtOAc/pet. ether step gradient gave 6.15 g (36%) of the title compound. ^1H NMR (DMSO-d6) δ1.40–1.46 (m, 2 H), 1.59–1.72 (m, 4 H), 1.78 (s, 3 H), 1.59–1.94 (m, 2 H), 2.16 (s, 3 H), 3.18 (quinet, 1 H), 3.81 (s, 2 H), 6.87 (d, 1 H), 7.06 (d, 2 H), 7.16–7.25 (m, 3 H), 7.46 (dd, 1 H), 7.55 (s, 1 H), 10.33 (s, 1 H).

Step 3

4-[4-(2-Benzyl-4.5-dimetbyl-furan-3-carbonyl)-2-cyclopentyl-phenoxysulfonyl]-2-hydroxy-benzoic acid

Tbe title compound was prepared according to tbe procedure in Example 4 using (2-benzyl4,5-dimethyl-furan-3-yl)-(3-cyclopentyl-4-hydroxy-phenyl)-methanone (0.509 g, 1.36 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.645 g, 2.72 mmol). Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with 20% EtOAc/hexane gave 0.430 g (55%) of the title compound a pale yellow solid, mp 80–85° C. ^1H NMR (DMSO-d6) δ1.23–1.34 (m, 2 H), 1.45–1.59 (m, 2 H), 1.61–1.74 (m, 4 H), 1.76 (s, 3 H), 2.16 (s, 3 H), 2.95 (quintet, 1 H), 3.78 (s, 2 H), 6.98 (d, 2 H), 7.13–7.25 (m, 4 H), 7.36–7.38 (m, 2 H), 7.44–7.57 (m, 2 H), 7.97 (d, 1 H). IR (KBr) 3400, 2950, 1690, 1580, 1480 and 1190 cm^{-1}. mass spectrum (–ESI) m/z 573 (M–H). Anal. Calcd. for $C_{32}H_{32}O_8S$. 0.2H_2O: C, 66.88; H, 5.26; N, 0.00. Found: C, 66.53; H, 5.40; N, 0.07.

EXAMPLE 18

4-[4-(2-Benzyl-4,5-dimethyl-thiophene-3-carbonyl)-2-cyclopentyl-phenoxysulfonyl]-2-hydroxy-benzoic acid

Step 1

2.3-Dimethylthiophene

A stirred mixture of commercial 3-methylthiophenecarboxaldehyde (20 g, 0.159 mol), hydrazine hydrate (31 mL) and diethylene glycol (72 mL) was refluxed for 20 min. After cooling below 100° C., potassium hydroxide (22.9 g, 0.408 mol) was slowly added and the reaction mixture was beated at 125–130° C. for 1.5 h. The reaction mixture was cooled to ambient temperature, quenebed with H_2O and extracted with ether. The combined ethereal extracts were washed with 5% aqueous HCl, brine, dried ($MgSO_4$) and concentrated. Purification on silica gel eluting with pentane provided the title compound as an oil (15.81 g, 89%): ^1H NMR (CDC13) δ6.97 (d, 1H, J=8 Hz), 6.77 (d, 1 H, J=8 Hz), 2.35 (s, 3 H), 2.14 (s, 3 H).

Step 2

2-Benzyl-4.5-dimethylthiophene

Tbe title compound was prepared according to the procedure in Example 5, step 1 using 2,3-dimetbylthiophene (5.00 g, 44.6 mmol), 2.5M BuLi/hexanes (17.9 mL, 44.6 mmol) and benzyl bromide (5.30 mL, 44.6 mmol) in THF (44.6 mL). Purification on Biotage KP-Sil elnting with 1% EtOAc/pet ether gave 6.96 g (77%) of the tide compound as an oil. ^1H NMR (DMSO-d6) δ2.01 (s, 3 H), 2.21 (s, 3 H), 3.98 (s, 2 H), 6.58 (s, 1 H), 7.18–7.37 (m, 5 H).

Step 3

(2-Benzyl-4,5-dimethyl-thiophen-3-yl)-(3-cyclopentyl-4-methoxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 2 using 3-cyclopentyl-p-anisic acid (5.0 g, 22.7 mmol, RN-59216-82-9), oxalyl chloride (2.4 mL, 27.2 mmol), N,N-dimethylformamide (2 drops) tin(IV) chloride (2.9 mL, 25.0 mmol) and 2-benzyl-2,3-dimethylthiophene (5.1 g, 25.0 mmol). Purification on silica gel eluting 5% EtOAc/pet. ether gave 4.8 g (52%) of the title compound as an amber oil. (DMSO-d6) δ7.61–7.54 (m, 2 H), 7.24–7.14 (m, 3 H), 7.08–7.02 (m, 3 H), 3.87 (s, 3 H), 3.84 (s, 2 H), 3.42–3.30 (m, 1 H), 2.26 (s, 3 H), 2.00–1.85 (m, 2 H), 1.81 (s, 3 H), 1.74–1.58 (m, 4 H), 1.48–1.36 (m, 2 H). mass spectrum (EI) m/z 404 (M+). Anal. Calc. for $C_{26}H_{28}O_2S$: C, 77.19, H, 6.98, N, 0.00. Found: C, 76.26, H, 7.24, N, 0.04.

Step 4

(2-Benzyl-4,5-dimethyl-thiophen-3-yl)-(3-cyclopentyl-4-hydrox-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 3 using (2-benzyl4,5-dimethyl-thiophen-3-yl)-(3-cyclopentyl-4-methoxy-phenyl)-metbanone (3.32 g, 8.20 mmol) and 1M boron tribromide/CH_2Cl_2 (1.6 mL) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with a 2, 5 & 10% EtOAc/hexane step gradient gave 0.63 g (18%) of the title compound. ^1H NMR (DMSO-d6) δ consistent.

Step 5

4-(2-Benzyl-4,5-dimetbyl-thiophene-3-carbonyl)-2-cyclopentyl-phenoxysulfonyl]-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 4 using (2-benzyl-4,5-dimethyl-thiophen-3-yl)-(3-cyclopentyl-4-hydroxy-phenyl)-methanone (0.505 g, 1.29 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.612 g, 2.58 mmol). Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with 15% EtOAc/hexane gave 0.432 g (57%) of the title compound a pale yellow solid, mp 70–77° C. ^1H NMR (DMSO-d6) δ1.29–1.34 (m, 2 H), 1.51–1.57 (m, 2 H), 1.62–1.77 (m,4 H), 1.79 (s, 3 H), 2.25 (s, 3 H), 2.99 (quintet, 1 H), 3.84 (s, 2 H), 6.98 (d, 2 H), 7.12–7.25 (m, 4 H), 7.39 (d, 2 H), 7.52 (dd, 1 H), 7.57 (d, 1 H), 7.99 (d, 1 H), IR (KBr) 3400, 2950, 1690, 1390 and 1190 cm^{-1}. mass spectrum (+ESI) m/z 591 (M+H). Anal. Calcd. for $C_{32}H_{30}O_7S_2.0.5H_2O$: C, 64.09; H, 5.21; N, 0.00. Found: C, 63.96; H, 5.30; N, 0.04.

EXAMPLE 19

4-[4-(2-Benzyl-4,5-dimethyl-furan-3-carbonyl)-2,6-diethyl-phenoxysulfonyl]-2-hydroxy-benzoic acid

Step 1

4-Bromo-2,6-diethylbenzenediazonium tetrafluoroborate

At 0° C., to a stirred solution of commercial 4-bromo-2,6-diethylaniline (10.0 g, 43.9 mmol) in absolute ethanol (219 mL) was added 48% aq. tetrafluoroboric acid (17.2 mL), followed by tert-butyl nitrile (5.7 mL, 48.0 mmol). After 0.5 h, the reaction was poured into ice cold ether (877 mL). The resulting precipitate was washed with cold ether and dried to give 9.39 g (66%) of the title compound. ^1H NMR: consistent.

Step 2

4-Bromo-2.6-diethylanisole

A stirred solution containing 4-bromo-2,6-diethylbenzenediazobium tetrafluoroborate (17.25 g, 52.77 mmol) and freshly ground anhydrous zinc chloride (7.2 g, 52.9 mmol) in metbanol (1.06 L) was refluxed for 6 h. The reaction was cooled to ambient temperature, quenched with H_2O (1.2 L), saturated with solid sodium chloride and extracted with hexane. The combined organic extracts were sequentially washed with sat. aq. $NaHCO_3$ (1×100 mL), with H_2O (1×100 mL), with brine (1×100 mL), dried (Na_2SO_4) and concentrated to give 12.0 g (94%) of tidle compound. ^{-1}H NMR: consistent. mass spectrum (EI), m/z 242 (M+).

Step 3

3.5-Diethyl-4-methoxybenzoic acid

At −78° C., to a stirred solution of 4-bromo-2,6-diethylanisole (12.0 g, 49.4 mmol) in THF (329 mL) was added dropwise n-butyllithium (27.2 mL, 43.5 mmol). After 3 b, the reaction was poured into crushed dry ice and allowed to warm to ambient temperature. The reaction mixture was diluted with EtOAc, concentrated, suspended in H_2O, acidified (pH 1), filtered and the collected solids washed with H_2O. The crude product was slurried in hexane (15 mL), collected by filtration and dried to give 6.76 g (66%) of the title compound. ^1H NMR: consistent. IR (KBr) consistent. mass spectrum (EI), m/z 208 (M+). Anal. Calcd. for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74; N, 0.00. Found: C, 69.28; H, 7.49, N, 0.07.

Step 4

(2-Benzyl-4.5-dimethyl-furan-3-yl)-(3.5-diethyl-4-methoxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 2, using 3,5-diethyl-4-methoxybenzoic acid (10.66 g, 51.3 mmol), oxalyl chloride (4.90 mL, 56.3 mmol), N,N-DMF (2 drops), tin TV chloride (6.60 mL, 56.3 mmol) and 2-benzyl-4,5-dimethylfuran (11.4 g, 61.3 mmol) to give 22.0 g, of the title compound. ^1H NMR δ1.13 (t, 6 H), 1.83 (s, 3 H), 2.19 (s, 3 H), 2.61 (q, 4 H), 3.74 (s, 3 H), 3.82 (s, 2 H), 7.05 (d, 2 H), 7.23–7.27 (m, 3 H), 7.42 (s, 2 H). mass spectrum (EI), m/z 376 (M+).

Step 5

(2-Benzyl-4,5-dimethyl-furan-3-yl)-(3.5-diethyl4-hydroxy-phenyl)-methanone

Tbe title compound was prepared according to the procedure in Example 5, step 3 using (2-benzyl-4,5-dimethyl-furan-3-yl)-(3,5-diethyl-4-methoxy-phenyl)-methanone (19.3 g, 51.2 mmol) and 1M boron tribromide/CH_2Cl_2 (36.8 mL) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with a 2 & 5% EtOAc/hexane step gradient gave 7.07 g (37%) of the title compound. ^1H NMR (DMSO-d6) δ1.08 (t, 6 H), 1.79 (s, 3 H), 2.17 (s, 3 H), 2.57 (q, 4 H), 3.81 (s, 2 H), 7.06 (dd, 2 H), 7.16–7.18 (m, 1 H), 7.22–7.26 (m, 2 H), 7.37 (s, 2 H), 9.10 (s, 1 H). mass spectrum (EI), m/z 362 (M+).

Step 6

4-[4-(2-Benzyl-4,5-dimethyl-furan-3-carbonyl)-2,6-diethyl-phenoxysulfonyl]-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 4 using (2-benzyl-4,5-dimethyl-furan-3-yl)-(3,5-diethyl-4-hydroxy-phenyl)-methanone (0.5 g, 1.38 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.65 g, 2.74 mmol). Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with 10% EtOAc/hexane gave 0.251 g (32%) of the title compound as an off white solid, mp 143–147° C. ^1H NMR (DMSO-d6) δ1.01 (t, 6 H), 1.84 (s, 3 H), 2.20 (s, 3 H), 2.51 (q, 4 H, with DMSO peak), 3.81 (s, 2 H), 7.01 (d, 2 H), 7.16–7.20 (m, 1 H), 7.23–7.27 (m, 2 H), 7.45 (s, 2 H), 7.49–7.53 (m, 2 H), 8.06 (d, 1 H). mass spectrum (−ESI), m/z 561 (M−H). Anal. Calcd. for $C_{32}H_{30}O_8S$: C, 66.18; H, 5.37; N, 0.00. Found: C, 65.80; H, 5.50; N, 0.22.

EXAMPLE 20

4-{4-[2-(4-Bromo-benzyl)4,5-dimethyl-thiopbene-3-carbonyl]-2-cyclopentylphenoxysulfonyl}-2-hydroxy-benzoic acid

Step 1

2-(4-Bromobenzyl)-4,5-dimethylthiophene

The tide compound was prepared according to the procedure in Example 5, step 1 using 2,3-dimethylthiophene (5.10 g, 45.5 mmol), 2.5M BuLi/hexanes (18.2 mL, 45.5 mmol) and 4-bromobenzyl bromide (11.4 g, 45.5 mmol) in THF. Purification on Biotage KP-Sil eluting with 100% pet ether gave 7.74 g (69%) of the title compound. ^{1}H NMR: consistent.

Step 2

[2-(4-Bromobenzyl)-4,5-dimethyl-thiophen-3-yl]-(3-cyclopentyl-4-methoxy-phenyl)-methanone

The tide compound was prepared according to the procedure in Example 5, step 2 using 3-cyclopentyl-p-anisic acid (3.50 g, 15.9 mmol, RN-59216-82-9), oxalyl chloride (1.52 mL, 17.5 mmol), N,N-dimethylformamide (2 drops) tin(I) chloride (2.04 mL, 17.5 mmol) and 2-(4-bromobenzyl)-4,5-dimethylthiophene (4.46 g, 15.9 mmol). Purification on silica gel eluting 5% EtOAc/pet. ether gave 4.05 g (53%) of the title compound as yellow oil. (DMSO-d6) δ1.43–1.47 (m, 2 H), 1.61–1.75 (m, 4 H), 1.81 (s, 3 H), 1.91–1.99 (m, 2 H), 2.28 (s, 3 H), 3.24 (quintet, 1 H), 3.84 (s,2 H), 3.88 (s, 3 H), 7.02 (d, 2 H), 7.06 (d, 1 H), 7.40 (d, 2 H), 7.52 (dd, 1 H), 7.57 (1 H).

Step 3

[2-(4-Bromobenzyl)-4,5-dimethyl-thiophen-3-yl]-(3-cyclopentyl-4-hydroxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 3 using [2-(4-bromobenzyl)4,5-dimethyl-thiophen-3-yl]-(3-cyclopentyl-4-methoxy-phenyl)-methanone (4.05 g, 8.39 mmol) and 1M boron tribromide/CH_2Cl_2(26.0 mL) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with 15% acetone/hexane gave 2.90 g (74%) of the title compound. (DMSO-d6) δ1.41–1.48 (m, 2 H), 1.59–1.74 (m, 4 H), 1.82 (s, 3 H), 1.89–1.96 (m, 2 H), 2.27 (s, 3 H), 3.20 (quintet, 1 H), 3.84 (s, 2 H), 6.88 (d, 1 H), 7.03 (d, 2 H), 7.39–7.43 (m, 3 H), 7.54 (d, 1 H), 10.47 (s, 1 H).

Step 4

4-{4-[2-(4-Bromo-benzyl)-4,5-dimethyl-thiophene-3-carbonyl]-2-cyclopentyl-phenoxysylfonyl}-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 4 using [2-(4-bromobenzyl)-4,5-dimethyl-thiophen-3-yl]-(3-cyclopentyl-4-hydroxy-phenyl)-methanone (1.01 g, 2.14 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (1.01 g, 4.29 mmol). Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with a 15 & 25% EtOAc/hexane step gradient followed by crystallization from ether/pet ether gave 0.549 g (38%) of the title compound as a tan solid, mp 165–170° C. ^{1}H NMR (DMSO-d6) δ1.22–1.32 (m, 2 H), 1.46–1.58 (m, 2 H), 1.61–1.76 (m, 4 H), 1.78 2.26 (s, 3 H), 2.97 (quintet, 1 H), 3.84 (s, 2 H), 6.94 (d, 2 H), 7.23 (d, 1 H), 7.34–7.40 (m, 4 H), 7.50 (dd, 1 H), 7.53 (d, 1 H), 8.00 (d, 1 H). IR (KBr) 3400, 2950, 1680, 1480, 1390 and 1190 cm^{-1}. mass spectrum (–ESI) m/z 667 (M–H). Anal. Calcd. for $C_{32}H_{29}BrO_7S_2$: C, 57.40; H, 4.37; N, 0.00. Found: C, 57.47; H, 4.24; N, 0.08.

EXAMPLE 21

4-{4-[2-(2-Bromo-benzyl)4,5-dimethyl-furan-3-carbonyl]-2-cyclopentyl-phenoxysulfonyl}-2-hydroxy-benzoic acid

Step 1

[2-(2-Bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3-cyclopentyl4-methoxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 2 using 3-cyclopentyl-p-anisic acid (2.00 g, 9.08 mmol, RN-59216–82–9), oxalyl chloride (0.871 mL, 9.99 mmol), N,N-dimethylformamide (2 drops), tin (IV) chloride (1.17 mL, 4.58 mmol) and 2-(2-bromo-benzyl)-4,5-dimethyl-furan (2.41 g, 9.08 mmol) in CH_2Cl_2. Purification on Biotage KP-Sil eluting with 4% EtOAc/pet. ether gave 2.82 g (66%) of the title compound as a clear oil. ^{1}H NMR (DMSO-d6) δ1.40–1.44 (m, 2 H), 1.57–1.73 (m, 4 H), 1.79 (s, 3 H), 1.87–1.93 (m, 2 H), 2.16 (s, 3 H), 3.21 (quintet, 1 H), 3.86 (s, 3 H)3.93 (s, 2 H), 7.04 (d, 1 H), 7.11–7.18 (m, 2 H), 7.29 (t, 1 H), 7.51 (dd, 1 H), 7.54–7.60 (m, 2 H).

Step 2

[2-(2-Bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3-cyclopentyl-4-hydroxy-phenyl)-methanone

The title compound was prepared according to the procedure in Example 5, step 3 using [2-(2-bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3-cyclopentyl-4-methoxy-phenyl)-methanone (2.82 g, 6.03 mmol) and 1M boron tribromide/CH_2Cl_2 (18.7 mL) in CH_2Cl_4. Purification on Biotage KP-Sil eluting with a 2, 5 & 10% EtOAc/pet. ether step gradient gave 1.36 g (50%) of the title compound. (DMSO-d6) δ1.42–1.44 (m, 2 H), 1.58–1.72 (m, 4 H), 1.79 (s, 3 H), 1.87–1.92 (m, 2 H), 2.16 (s, 3 H), 3.17 (quintet, 1 H), 3.92 (s, 2 H), 6.85 (d, 1 H), 7.12–7.17 (m, 2 H), 7.29 (t, 1 H), 7.45 (dd, 1 H), 7.50–7.52 (m, 2 H), 10.31 (s, 1 H).

Step 3

4-{4-[2-(2-Bromo-benzyl)4,5-dimethyl-furan-3-carbonyl]-2-cyclopentyl-pbenoxysulfonyl}-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 4 using [2-(2-bromo-benzyl)-4,5-dimethyl-furan-3-yl]-(3-cyclopentyl-4-methoxy-phenyl)-methanone (0.516g, 1.14 mmol) and 4-chlorosulphonyl-2-hydroxybenzoic acid (0.540 g, 2.28 mmol). Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with a 15 & 25% EtOAc/hexane step gradient gave 0.509 g (68%) of the title compound as a brown solid, mp 72–82° C. ^{1}H NMR (DMSO-d6) δ1.25–1.34 (m, 2 H), 1.50–1.55 (m, 2 H), 1.62–1.74 (m, 4 H), 1.77 (s, 3 H), 2.15 (s, 3 H), 2.95 (quintet, 1 H), 3.88 (s, 2 H), 7.10–7.15 (m, 2 H), 7.20 (d, 1 H), 7.27 (t, 1 H), 7.35–7.37 (m, 2 H), 7.48–7.58 (m, 3 H), 7.96 (dd, 1 H). IR (KBr) 3400, 2950, 1690, 1390 and 1190 cm^{-1}. mass spectrum (–ESI) n/z 651 (M–H). Anal. Calcd. for $C_{32}H_{35}BrO_8S$. 0.4H_2O: C, 58.17; H, 4.55; N, 0.00. Found: C, 58.20; H, 4.55; N, 0.03.

EXAMPLE 22

2-Acetoxy4-[4-(2-benzyl-4,5-dimethyl-furan-3-carbonyl)-2-cyclopentyl-phenoxysulfonyl]-benzoic acid

A stirred solution containing 4-[4-(2-benzyl-4,5-dimethyl-furan-3-carbonyl)-2-cyclopentyl-phenoxysulfonyl]-2-hydroxy-benzoic acid (0.235 g, 0.409 mmol), acetic anhydride (3.3 mL) and magnesium iodide (0.113 g, 0.409 mmol) in ether (8 mL) was refluxed for 0.5 h. The reaction was allowed to cool to ambient temperature, quenched with H_2O (100 mL), extracted with ether and

concentrated. The mixed anhydride/product was dissolved in THF (6 mL), treated with H_2O (6 mL) and refluxed for 0.5 h. The reaction was allowed to cool to ambient temperature, quenched with H_2O (100 mL), extracted with ether and concentrated. Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with a 18 & 25% EtOAc/pet ether step gradient followed by crystallization from acetone/hexane gave 0.201 g (60%) of the title compound as an off white solid, mp 55–90° C. ^1H NMR (DMSO-d6) δ1.23–1.32 (m, 2 H), 1.50–1.53 (m, 2 H), 1.64–1.72 (m, 4 H), 1.76 (s, 3 H), 2.17 (s, 3 H), 2.23 (s, 3 H), 2.92 (quintet, 1 H), 3.79 (s, 2 H), 6.98–7.00 (m, 2 H), 7.15–7.23 (m, 4 H), 7.53 (dd, 1 H), 7.58 (d, 1 H), 7.84 (d, 1 H), 7.88 (dd, 1 H), 8.12 (d, 1 H) 13.7 (br s, 1 H). mass spectrum (–ESI) m/z 615 (M–H). Anal. Calcd. for $C_{33}H_{32}O_9S \cdot 0.5H_2O$: C, 65.27; H, 5.32; N, 0.00. Found: C, 65.33; H, 5.30; N, 0.03.

EXAMPLE 23

2-Acetoxy4-[4-(2-benzyl-4,5-dimethyl-thiophene-3-carbonyl)-2-cyclopentyl-phenoxysulfonyl]-benzoic acid

The title compound was prepared according to the procedure in Example 22 using 4-[4-(2-benzyl4,5-dimethyl-thiophene-3-carbonyl)-2-cyclopentyl-phenoxysulfonyl]-2-hydroxy-benzoic acid (0.233 g, 0.394 mmol), magnesium iodide (0.110 g, 0.394 mmol) and acetic anhydride (3.3 mL) in ether. Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with 18% EtOAc/pet ether followed by a second chromatography on 2% H_3PO_4/MeOH treated silica gel, eluting with 5% CH_3CN/CH_2Cl_2 gave 0.128 g (51%) of the title compound as a white solid, mp 94–96° C. ^1H NMR (DMSO-d6) δ1.24–1.33 (m, 2 H), 1.52–1.57 (m, 2 H), 1.61–1.76 (m, 4 H), 1.79 (s, 3 H), 2.23 (s, 3 H), 2.26 (s, 3 H), 2.94 (quintet, 1 H), 3.85 (s, 2 H), 6.99–7.01 (m, 2 H), 7.13 (m, 4 H), 7.52 (dd, 1 H), 7.59 (d, 1 H), 7.84 (d, 1 H), 7.90 (dd, 1 H), 8.14 (d, 1 H), 13.8 (br s, 1 H). mass spectrum (–ESI) m/z 631 (M–H). Anal. Calcd. for $C_{34}H_{32}O_9S_2O \cdot 0.2H_2O$: C, 64.17; H, 5.13; N, 0.00. Found: C, 64.06; H, 4.98; N, 0.04.

EXAMPLE 24

2-Acetoxy-4-{4-[2-(4-Bromo-benzyl)-4,5-dimethyl-thiophene-3-carbonyl]-2-cyclopentyl-phenoxysulfonyl}-benzoic acid

The title compound was prepared according to the procedure in Example 22 using 4-{4-[2-(4-bromo-benzyl)-4,5-dimethyl-thiophene-3-carbonyl]-2-cyclopentyl-phenoxysulfonyl}-2-hydroxy-benzoic acid (0.211 g, 0.315 mmol), magnesium iodide (0.0876 g, 0.315 mmol) and acetic anhydride (2.5 mL) in ether. Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with a 10 & 20% EtOAc/pet ether step gradient followed by crystallization from acetone/hexane gave 0.185 g (57%) of the tide compound as a white solid, mp 113–114° C. ^1H NMR (DMSO-d6) δ1.27–1.34 (m, 2 H), 1.51–1.58 (m, 2 H), 1.63–1.78 (m, 4 H), 1.80 (s, 3 H), 2.25 (s, 3 H), 2.28 (s,3 H), 2.95 (quintet, 1 H), 3.86 (s, 2 H), 6.97 (d, 2 H), 7.24 (d, 1 H), 7.38 (d, 2 H) 7.52 (dd, 1 H), 7.57 (d, 1 H), 7.86 (d, 1 H), 7.92 (dd, 1 H), 8.16 (d, 1 H) 13.8 (br,s 1 H). mass spectrum (–ESI) m/z 709 (M–H). Anal. Calcd. for $C_{33}H_{31}BrO_9S_2$: C, 57.38; H, 4.39; N, 0.00. Found: C, 56.99; H, 4.38; N, 0.02.

EXAMPLE 25

2-Acetoxy-4-{4-[2-(2-Bromo-benzyl)-4,5-dimethyl-fuiran-3-carbonyl]-2-cyclopentyl-phenoxysulfonyl}-benzoic acid

The title compound was prepared according to the procedure in Example 22 using 4-{4-[2-(2-hromo-benzyl)-4,5-dimethyl-furan-3-carbonyl]-2-cyclopentyl-phenoxysulfonyl}-2-hydroxy-benzoic acid (0.318 g, 0.487 mmol), magnesium iodide (0.132 g, 0.475 mmol) and acetic anhydride (3.8 mL) in ether. Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with a 30% EtOAc/pet ether gave 0.083 g (25%) of the title compound as a solid, mp 79° C. ^1H NMR (DMSO-d6) δ1.27–1.33 (m, 2 H), 1.51–1.54 (m, 2 H), 1.63–1.73 (m, 4 H), 1.79 (s, 3 H), 2.17 (s, 3 H), 2.24 (s, 3 H), 2.93 (quintet, 1 H), 3.91 (s, 2 H), 7.13–7.17 (m, 2 H), 7.21 (d, 1 H), 7.30 (dt, 1 H), 7.51 (dd, 1 H), 7.55 (dd, 1 H), 7.60 (d, 1 H), 7.85 (d, 1 H), 7.90 (dd, 1 H), 8.14 (d, 1 H)13.8 (br s, 1 H). mass spectrum (–ESI) m/z 693/695 (M–H). Anal. Calcd. for $C_{34}H_{33}BrO_9S$: C, 58.71; H, 4.49; N, 0.00. Found: C, 58.04; H, 4.52; N, 0.04.

EXAMPLE 26

2-Acetoxy-4-{4-[2-(2-butyl-benzofuran-3-yl)-ethyl]-phenoxysulfonyl}-benzoic acid

At ambient temperature, to a stirred solution containing 4-{4-[2-(2-hutyl-henzofuran-3-yl)-ethyl]-phenoxysulfonyl}-2-hydroxy-benzoic acid (0.298 g, 0.602 mmol) and acetic anhydride (1 mL) In EtOAc (2 mL) was added 4-(dimethylamino)pyridine (3.7 mg, 0.0301 mmol). The reaction was refluxed for 72 h. The reaction was cooled to ambient temperature, hydrolyzed with H_2O (5 mL) and stirred for 24 h. The reaction was diluted with EtOAc and the organic phase washed sequentially with H_2O (2×), brine (2×), dried ($MgSO_4$) and concentrated. Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with a 10 & 20% EtOAc/pet ether step gradient gave 0.122 g (76%) of the title compound as a yellow solid, mp 54–57° C. ^1H NMR consistent. IR (KBr) 3400, 2900, 1700, 1380 and 1190 cm^{-1}. mass spectrum (–ESI) m/z 535 (M–H).

EXAMPLE 27

1-Methyl-1H-pyrazole-4-sulfonic acid 4-[2-(4-bromo-benzyl)-4,5-dimethyl-thiophene-3-carbonyl]-2-cyclopentyl-phenyl ester

Step 1

1-Methyl-1H-pyrazole4-sulfonyl chloride

At 5° C., to stirred chlorosulfonic acid (81.0 mL, 1.22 mol) was cautiously added N-methylpyrazole (25.0 g, 0.304 mol) over a 1 h period. After the addition was complete the reaction was stirred at ambient temperature for 0.5 h, then heated at 110° C. for 16 h. At 5° C., to the reaction was added dropwise thionyl chloride (55.0 mL, 0.754 mol). After the addition was complete the reaction was stirred at ambient temperature for 1 h, then heated to reflux for 2 h. The reaction was cooled to 15° C. and carefully quenched (dropwise) into crushed ice. The title compound was collected by filtration and dried in vacuo to give 37.2 g (68%) of a white solid, mp 51° C. ^1H NMR ($CDCl_3$) δ4.00 (s, 3 H), 7.95 (s, 1 H), 8.03 (s, 3 H). IR (KBr) 3125, 1700, 1520, 1400 and 1370 cm^{-1}. mass spectrum (EI) m/z 180 (M+). Anal. Calcd. for $C_4H_5ClN_2O_2S$: C, 26.60; H, 2.79; N, 15.51. Found: C, 26.25; H, 2.45; N, 15.16.

Step 2

1-Methyl-1H-pyrazole4-sulfonic acid 4-[2-(4-bromo-benzyl)-4,5-dimethyl-thiophene-3-carbonyl]-2-cyclopentyl-phenyl ester

At ambient temperature, to a stirred solution of [2-(4-bromobenzyl)-4,5-dimethyl-thiophen-3-yl]-(3-cyclopentyl4-hydroxy-phenyl)-methanone (0.283 g, 0.603 mmol) in N,N-DMF (3.0 mL) was added 60% sodium hydride/mineral oil (24.1 mg, 0.603 mmol). After 0.5 h, to

the reaction was added a solution of 1-methyl-1H-pyrazole-4-sulfonyl chloride (0.120 g, 0.664 mmol) in N,N-DNF (1.3 mL). After 3 h, the reaction was quenched with 1N NaOH (40 mL), extracted with CH_2Cl_2, dried (Na_2SO_4) and concentrated. Purification on Biotage KP-Sil eluting with 20% acetone\hexane gave 0.202 g (55%) of the title compound as a yellow solid, mp 55–60° C. (DMSO-d6) δ1.29–1.34 (m, 2 H), 1.59–1.79 (m, 6 H), 1.81 (s, 3 H), (s, 3 H), 3.08 (quintet, 1 H), 3.87 (s, 2 H), 3.89 (s, 3 H). 6.98 (d, 2 H), 7.32 (d, 1 H), 7.39 (d, 2 H), 7.53–7.57 (m, 2 H), 7.97 (s, 1 H), 8.62 (s, 1 H). IR (KBr) 3450, 2950, 1650, 1490, 1400, 1370 and 1170 cm^{-1}. mass spectrum (+ESI) m/z 613/615 (M +H).

EXAMPLE 28

4-[4-(2-Butyl-benzofuran-3-ylmethyl)-phenoxysulfonyl]-2-hydroxy-benzoic acid

Step 1

4-[(2-Butyl-benzofuran-3-yl)-hydroxy-methyl]-phenol

A solution containing commercial 2-n-butyl-3-(4-hydroxybenzoyl)benzofuran (0.500 g, 17.0 mmol) and lithium aluminum hydride (34.0 mmol) in THF (84 mL) was refluxed for 3 h. The reaction was cooled to −10° C., and carefully quenched with 0.1N NaOH and H_2O. The aqueous solution was extracted with ether and concentrated. Purification on silica gel gave the title compound as a white solid, mp 94–96° C. (DMSO-d6) δ0.90 (t, 3 H), 1.35 (sextet, 2 H), 1.64 (quintet, 2 H), 2.84 (t, 2 H), 5.6 (d, 1 H), 5.87 (d, 1 H), 6.69 (d, 2 H), 7.07 (t, 1 H), 7.15 (dt, 1 H), 7.22 (d, 2 H), 7.40–7.43 (m, 2 H), 9.24 (s, 1 H). IR (KBr) 3350, 2950, 1610, 1510 and 1450 cm^{-1}. mass spectrum (EI) m/z 296 (M+). Anal. Calcd. for $C_{19}H_{20}O_3$ C, 77.00; H, 6.80; N, 0.00. Found: C, 75.63; H, 6.86; N, 0.04.

Step 2

4-(2-Butyl-benzofuran-3-ylmethyl)-phenol

At −10° C., to a stirred solution of 4-[(2-butyl-benzofuran-3-yl)-hydroxy-methyl]-phenol (2.78 g, 9.39 mmol) in CH_3CN was added portionwise triethylsilane (two ×1.50 mL, 18.78 mmol total, @ 0.5 h interval). To the reaction was added $BF_3 \cdot Et_2O$ (1.19 mL, 9.39 mmol) and the reaction was stirred for 10 min. The reaction was quenched with sat. aq. K_2CO_3 and extracted with CH_2Cl_2. The organic extracts were washed with brine (3×), dried ($MgSO_4$) and concentrated. Purification on silica gel gave 2.35 g (89%) the title compound as a white solid, mp 67–70° C. (DMSO-d6) δ0.87 (t, 3 H), 1.31 (sextet, 2 H), 1.62 (quintet, 2 H), 2.78 (t, 2 H), 3.84 (s, 2 H), 6.64 (d, 2 H), 7.02 (d, 2 H), 7.09 (dt, 1 H), 7.16 (dt, 1 H), 7.31 (d, 1 H), 7.42 (d, 1 H), 9.16 (s, 1 H). IR (KBr) 3300, 2950, 1610, 1510 and 1450 cm^{-1}. mass spectrum (EI) mz 280M +).

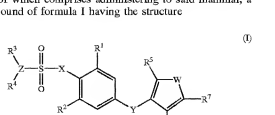
Step 3

4-[4-(2-Butyl-benzofuran-3-ylmethy)-phenoxysulfonyl]-2-hydroxy-benzoic acid

The title compound was prepared according to the procedure in Example 4 using 4-(2-butyl-benzofuran-3-ylmethyl)-phenol (0.500 g, 1.78 mmol) and 4-chlorosulphonyl -2-hydroxybenzoic acid (0.844 g, 3.56 mmol). Purification on 2% H_3PO_4/MeOH treated silica gel, eluting with EtOAc/hexane gave 0.728 g (86%) of the title compound as an orange solid, mp 135–138° C. ^1H NMR (DMSO-d6) δ0.84 (t, 3 H), 1.27 (sextet, 2 H), 1.58 (quintet, 2 H), 2.76 (t, 2 H), 3.97 (s, 2 H), 6.96 (d, 2 H) 7.10 (dt, 1 H), 7.17 (dt, 1 H), 7.22–7.31 (m, 5 H), 7.43 (d, 1 H), 7.95 (d, 1 H). IR (KBr) 3300,2950, 1675, 1390 and 1175 cm^{-1}. mass spectrum (−ESI) m/z 479 (M−H). Anal. Calcd. for $C_{26}H_{24}O_5S$ 0.2H_2O: C, 64.50; H, 5.08; N, 0.00. Found: C, 64.50; H, 5.07; N, 0.06.

What is claimed is:

1. A method of treating metabolic disorders mediated by insulin resistance or hyperglycemia in a mammal in need thereof which comprises administering to said mammal, a compound of formula I having the structure



(I)

wherein

R^1 and R^2 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, halogen, perfluoroalkyl of 1–6 carbon atoms, cycloalkyl of 3–8 carbon atoms, thienyl, furyl, phenyl or phenyl substituted with trifluoromethyl, chloro, methoxy, or trifluoromethoxy;

R^3 and R^4 are each, independently, hydrogen, carboxyl, hydroxyl, hydoxyalkyl of 1–6 carbon atoms, alkoxy of 1–6 carbon atoms, perfluoroalkoxy of 1–6 carbon atoms, alkanoyloxy of 2–7 carbon atoms, perfluoroalkanoyloxy of 2–7 carbon atoms, arylalkoxy of 7–15 carbon atoms, aryloxy of 6–12 carbon atoms, aroyloxy of 6–12 carbon atoms, aryloxycarbonyl of 7–13 carbon atoms, alkoxycarbonyl of 2–7 carbon atoms, perfluoroalkoxycarbonyl of 2–7 carbon atoms, alkyl of 1–6 carbon atoms, perfluoroalkyl of 1–6 carbon atoms, alkylamino of 1–6 carbon atoms, dialkylamino of 1–6 carbon atoms per alkyl group, tetrazolyl mercapto, nitrile, nitro, amino, —$NHSO_2CF_3$, carbamoyl, carboxyaldehyde, halogen, acylamino, 3-hydroxy-cyclobut- 3-ene-4-yl-1,2-dione, pyridyl, isoxazolyl, pyrimidyl or pyrimidyl substituted with mercapto, or tetronic acid;

R^5 is hydrogen, alkyl of 1–6 carbon atoms, perfluoroalkyl of 1–6 carbon atoms, naphthalenylmethyl, henzyl or henzyl substituted with halogen,

R^6 and R^7 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, or perfluoroalkyl of 1–6 carbon atoms, or R^6 and R^7 may be taken together as a diene unit having the structure —CH=CH—CH=CH—;

W is S or O

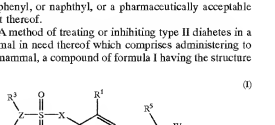
X is —NR^8CH_2—, —NR^8—, or O;

R^8 is hydrogen or alkyl;

Y is carbonyl, methylene, ethyl, or —$NHCH_2$—;

Z is phenyl, or naphthyl, or a pharmaceutically acceptable salt thereof.

2. A method of treating or inhibiting type II diabetes in a mammal in need thereof which comprises administering to said mammal, a compound of formula I having the structure



(I)

wherein

R^1 and R^2 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, halogen, perfluoroalkyl of 1–6 carbon atoms, halogen, perfluoroalkyl of 1–6 carbon

atoms, cycloalkyl of 3–8 carbon atoms, thienyl, furyl, phenyl or phenyl substituted with trifluoromethyl, chloro, methoxy, or trifluoromethoxy;

R^3 and R^4 are each, independently, hydrogen, carboxyl, hydroxyl, hydoxyalkyl of 1–6 carbon atoms, alkoxy of 1–6 carbon atoms, alkoxy of 1–6 carbon atoms, perfluoroalkoxy of 1–6 carbon atoms, alkanoyloxy of 2–7 carbon atoms, perfluoroalkanoyloxy of 2–7 carbon atoms, arylalkoxy of 7–15 carbon atoms, aryloxy of 6–12 carbon atoms, aroyloxy of 6–12 carbon atoms, aryloxycarbonyl of 7–13 carbon atoms, alkoxycarbonyl of 2–7 carbon atoms, perfluoroalkoxycarbonyl of 2–7 carbon atoms, alkyl of 1–6 carbon atoms, perfluoroalkyl of 1–6 carbon atoms, alkylamino of 1–6 carbon atoms, dialkylamino of 1–6 carbon atoms per alkyl group, tetrazolyl, mercapto, nitrile, nitro, amino, $—NHSO_2CF_3$, carbamoyl, carboxyaldehyde, halogen, acylamino, or 3-hydroxy-cyclobut-3-ene-4-yl-1,2-dione, pyridyl, isoxazolyl, pyrimidyl or pyrimidyl substituted with mercapto, or tetronic acid;

R^5 is hydrogen, alkyl of 1–6 carbon atoms, perfluoroalkyl of 1–6 carbon atoms, naphthalenylmethyl, benzyl or benzyl substituted with halogen,

R^6 and R^7 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, or perfluoroalkyl of 1–6 carbon atoms, or R^6 and R^7 may be taken together as a diene unit having the structure $—CH═CH—CH═CH—$;

W is S or O,

X is $—NR^8CH_2—$, $—NR^8—$, or O;

R^8 is hydrogen or alkyl;

Y is carbonyl, methylene, ethyl, or $—NHCH_2—$;

Z is phenyl, naphthyl, pyrazolyl, isoxazolyl, or isothiazolyl; or a pharmaceutically acceptable salt thereof.

3. A method of modulating glucose levels in a mammal in need thereof which comprises administering to said mammal, a compound of formula I having the structure

(I)

wherein

R^1 and R^2 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, halogen, perfluoroalkyl of 1–6 carbon atoms, cycloalkyl of 3–8 carbon atoms, thienyl, furyl, phenyl or phenyl substituted with trifluoromethyl, chloro, methoxy, or trifluoromethoxy;

R^3 and R^4 are each, independently, hydrogen, carboxyl, hydroxyl, hydoxyalkyl of 1–6 carbon atoms, alkoxy of 1–6 carbon atoms, perfluoroalkoxy of 1–6 carbon atoms, alkanoyloxy of 2–7 carbon atoms, perfluoroalkanoyloxy of 2–7 carbon atoms, arylalkoxy of 7–15 carbon atoms, aryloxy of 6–12 carbon atoms, aroyloxy of 6–12 carbon atoms, aryloxycarbouyl of 7–13 carbon atoms, alkoxycarbonyl of 2–7 carbon atoms, perfluoroalkoxycarbonyl of 2–7 carbon atoms, alkyl of 1–6 carbon atoms, perfluoroalkyl of 1–6 carbon atoms, alkylamino of 1–6 carbon atoms, dialkylamino of 1–6 carbon atoms per alkyl group, tetrazolyl, mercapto, nitrile, nitro, amino, $—NHSO_2CF_3$, carbamoyl, carboxyaldehyde, halogen, acylamino, or 3-hydroxy-cyclobut-3-ene-4-yl-1,2-dione, pyridyl, isoxazolyl, pyrimidyl or pyrimidyl substituted with mercapto, or tetronic acid;

R^5 is hydrogen, alkyl of 1–6 carbon atoms, perfluoroalkyl of 1–6 carbon atoms, naphthalenylmethyl, benzyl or benzyl substituted with halogen,

R^6 and R^7 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, or perfluoroalkyl of 1–6 carbon atoms, or R^6 and R^7 may be taken together as a diene unit having the structure $—OH═CH—CH═CH—$;

W is S or 0,

X is $—NR^8CH_2—$, $—NR^8—$, or O;

R^8 is hydrogen or alkyl;

Y is carbonyl, methylene, ethyl, or $—NHCH_2—$;

Z is phenyl, naphthyl, isoxazolyl, or isothiazolyl;

or a pharmaceutically acceptable salt thereof.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

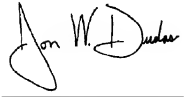
PATENT NO. : 6,683,107 B2
DATED : January 27, 2004
INVENTOR(S) : Paul J. Dellings et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,
Item [62], **Related U.S. Application Priority Data**,
"09/564,469" should read -- 09/564,496 --; and
"May 10, 2000" should read -- May 10, 1999 --

Signed and Sealed this

Fourteenth Day of December, 2004



JON W. DUDAS
Director of the United States Patent and Trademark Office

Exhibit F

References and Notes

(1) Presented in part at the 172nd National Meeting of the American Chemical Society, San Francisco, 1976.
(2) (a) Robert A. Welch Postgraduate Fellow; (b) taken in part from the Master's Thesis of D.M.C., Trinity University, 1970.
(3) (a) NSF Undergraduate Research Participant; (b) Robert A. Welch Undergraduate Scholar.
(4) The interesting photochemical properties of acenaphthylene may be found in references in the following papers: (a) W. I. Ferree, Jr., B. F. Plummer, and W. W. Schloman, Jr., *J. Am. Chem. Soc.*, **96**, 7741 (1974); (b) D. O. Cowan and J. Kozlar, *ibid.*, **98**, 1001 (1976).
(5) J. R. Erdman and H. E. Simmons *J. Org. Chem.*, **33**, 3808 (1968).
(6) It has not escaped our attention that compound 3 offers potential for the storage of solar energy[7] and we are continuing our efforts to find suitable photochemical conditions for its transformation.
(7) (a) G. Jones and B. R. Ramachandran, *J. Org. Chem.*, **41**, 798 (1976). (b) D. P. Schwendeman and C. Kutal, *Inorg. Chem.*, **16**, 719 (1977).
(8) (a) D. R. Adams and D. I. Davies, *J. Chem. Soc., Perkin Trans. 1*, 2012, (1974); (b) *ibid.*, 1237, (1972).
(9) D. C. Morrison, *J. Org. Chem.*, **25**, 1665 (1960).
(10) The assignment of endo orientation to 1 is based upon the classical Alder Rule[11] and the theoretical framework of Woodward–Hoffmann formalism.[12]
(11) K. Alder and G. Stein, *Angew. Chem.*, **50**, 514 (1937).
(12) R. B. Woodward and R. Hoffmann, "The Conservation of Orbital Symmetry", Academic Press, New York, N.Y., 1970.
(13) J. H. Beynon, "Mass Spectrometry and Its Applications to Organic Chemistry", Elsevier, Amsterdam, 1960.
(14) J. Michl, *J. Am. Chem. Soc.*, **96**, 4546 (1976).
(15) A full spectrum was not reported so that the extent of the perturbation of the K band is unknown: K. Yernemotao, M. Morioka, and I. Murata, *Tetrahedron Lett.* 3009 (1975).
(16) M. N. Paddon-Row, D. N. Butler, and R. N. Warrener, *J. Chem. Soc., Chem. Commun.* 741 (1976).
(17) The diagrams we have chosen for illustration are arbitrarily illustrated with the alkoxy group in the exo position for clarity of presentation. We shall attempt to identify the correct geometry for these adducts when subsequent studies are finished.
(18) (a) M. Akhtar, D. M. Bratby, J. C. Chadwick, and G. I. Fray, *Tetrahedron*, **32**, 2265 (1976). (b) L. S. Besford, R. C. Cookson, and J. Cooper, *J. Chem. Soc. C*, 1365 (1967).
(19) R. Baker and T. J. Mason, *J. Chem. Soc. C*, 598 (1970).
(20) K. L. Williamson, Y. Hsu, and E. I. Young, *Tetrahedron*, **24**, 5007 (1968).
(21) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed, Pergamon, New York, N.Y., 1969, p 334–344.
(22) Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Infrared spectra were recorded in KBr pellets on a Perkin-Elmer 337 or 283. NMR spectra were obtained in dilute solutions of $CDCl_3$ or CCl_4 with internal Me_4Si on a Varian T-60. UV–visible spectra were obtained on a Cary 118C. GLC analyses were run on a Varian Hy-Fi 2400 with a flame-ionization detector and a 5 ft × 0.125 in. column of 1.8% QV-101 on Chromosorb G. Mass spectra were recorded on a Finnegan 1015 C system/150 Quadrupole spectrometer at 70 eV. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Versatile Allene and Carbon Dioxide Equivalents for the Diels–Alder Reaction

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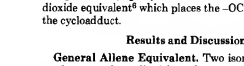
Received May 10, 1977

The Diels–Alder cycloaddition of vinyltriphenylphosphonium bromide (4) with a variety of 1,3-dienes generated the unsaturated cyclic phosphonium salts 3 in excellent yield. Wittig condensation of the ylides of 3 with aldehydes afforded the alkylidene derivatives. In addition, the known Diels–Alder adducts 13 were prepared from diethyl ketomalonate (12) and 1,3-dienes. These dihydropyrans could be transformed, via diacids.14, to β,γ-unsaturated valerolactones 15 by either lead tetraacetate mediated oxidative decarboxylation or by the Curtius degradation.

The Diels–Alder reaction figures prominently in the arsenal of organosynthetic reactions, and a wealth of knowledge exists concerning reactivity profiles, regioselectivity, and stereochemistry of the 4 + 2 cycloaddition reaction.[2] In recent years the construction of synthetic equivalents for unreactive dienophiles such as ketene[3] and allene[4] has extended the scope of this cyclization reaction to the production of cyclohexene systems not normally generated by this thermal process. This report relates the development of two such Diels–Alder equivalents: an allene equivalent[5] capable of introducing the $-CH_2C(=CHR)-$ group in a Diels–Alder sense, and a carbon dioxide equivalent[6] which places the $-OC(=O)-$ group into the cycloadduct.

Results and Discussion

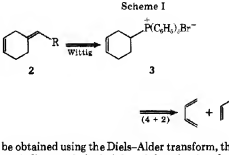
General Allene Equivalent. Two isomeric Diels–Alder products may be realized from the 4 + 2 cyclization between alkyl-substituted allenes and 1,3-dienes (eq 1).[7] It was felt that



1 2 (1)

an allene equivalent capable of producing the alkylidene moiety in 2 might be obtained from the intermediate 3 via a Wittig transformation (Scheme I). Cycloadduct 3 might then

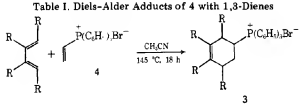
Scheme I



be obtained using the Diels–Alder transform, thus requiring a 1,3-diene and vinyltriphenylphosphonium bromide (4) as starting materials.

Indeed, vinyltriphenylphosphonium bromide[8] underwent smooth Diels–Alder reaction with a number of dienes at elevated temperatures to afford the desired adducts in excellent yield as shown in Table I. Cycloadducts 3a–e were recovered as powders after recrystallization. These new phosphonium salts could be readily converted to the ylides by treatment with lithium diisopropylamide at −78 °C in tetrahydrofuran. Addition of a slight excess of aldehyde at 0 °C followed by warming to room temperature afforded the alkylidene derivatives as shown in Table II. Formaldehyde, aliphatic and aromatic aldehydes, and α,β-unsaturated aldehydes underwent condensation and the desired olefins were obtained in good yield although, in some cases, product volatility con-

Table I. Diels–Alder Adducts of 4 with 1,3-Dienes



4 3

Registry no.	Diene	Adduct	Yield,[a] %
106-99-0	1,3-Butadiene	3a	93
78-79-5	Isoprene	3b	90[b]
513-81-5	2,3-Dimethyl-1,3-butadiene	3c	92
542-92-7	Cyclopentadiene	3d	90[c]
592-57-4	1,3-Cyclohexadiene	3e	96[c]

[a] Yields are reported after recrystallization from $CHCl_3$–Et_2O. [b] The para isomer was found in greater than 90% excess over the meta isomer. See ref 14. [c] Both ^1H and ^{31}P NMR methods failed to allow analysis of endo:exo isomer ratio. ^{13}C NMR spectrometry did reveal a ratio of approximately 80:20; however, exact structure assignment was not possible.

tributed to substantial decreases in the actual quantity isolated.

It is well documented that allenes possessing electron-withdrawing groups undergo Diels–Alder reactions in generally good yield across the α,β portion of the allenic π-bond system.[7b] Condensation of the ylide of the bicyclic phosphonium salt **3d** with glyoxal monodiethyl acetal[8] led to isolation of a mixture of *Z* and *E* isomers of the conjugated aldehyde **6** (required for another study) following treatment of the crude acetal Wittig product with silica gel in pentane (eq 3). Mild

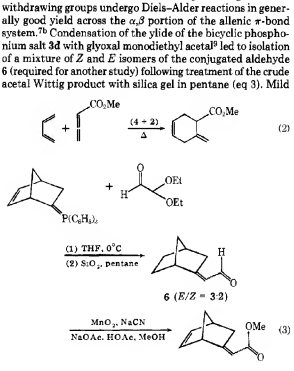
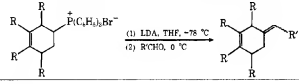


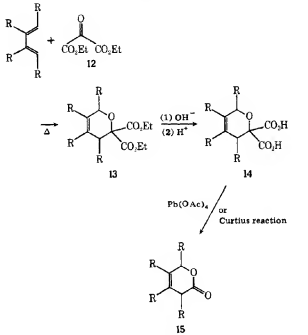
Table II. Wittig Condensations of 3a–e with Aldehydes



Registry no.	Phosphonium salt	Registry no.	Aldehyde	Product[a]	Yield,[b] %
63797-62-6	3a	100-52-7	C_6H_5CHO	5a	78
63797-63-7	3b		C_6H_5CHO	5b	80
54222-64-9	3c		C_6H_5CHO	5c	75
	3d		C_6H_5CHO	5d	81
	3d	5344-23-0	$(C_6H_5O)_2CHCHO$	6 (E) = 60% (Z) = 40%	35
	3e		C_6H_5CHO	5e	85
	3e	50-00-0	CH_2O(g) or $(CH_2O)_n$	7	50
	3e	111-71-7	n-$C_6H_{13}CHO$	8	63
	3e	4170-30-3	$CH_3CH{=}CHCHO$	9	30

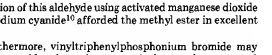
[a] Amounts of *Z* and *E* isomers were not determined except in 6. [b] Yields are reported after chromatographic purification.

Scheme II



oxidation of this aldehyde using activated manganese dioxide and sodium cyanide[10] afforded the methyl ester in excellent yield.

Furthermore, vinyltriphenylphosphonium bromide may also be considered as a ketene equivalent as demonstrated below using **3e** (eq 4). By bubbling a stream of oxygen through



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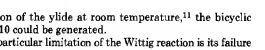
a solution of the ylide at room temperature,[11] the bicyclic ketone **10** could be generated.

One particular limitation of the Wittig reaction is its failure to allow formation of tetrasubstituted olefins.[12] For example,



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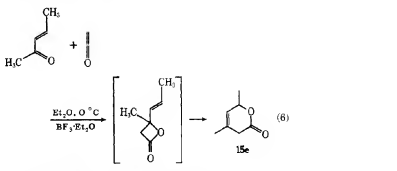
an attempt at the synthesis of the natural product terpinolene[13] **11** by condensation of acetone with the ylide of **3b** led to recovery of starting material after 24 h.

Carbon Dioxide Equivalent. To complete the triad of Diels–Alder equivalents for carbon and oxygen cumulated systems, a study was undertaken to develop a method for the introduction of the $-OC(=O)-$ group into the 4 + 2 cycloadduct. Of the myriads of known carbonyl compounds, only a few have been shown to act as dienophiles in the Diels–Alder reaction.[15] Diethyl ketomalonate[8] (**12**) is one such species, and its Diels–Alder adducts with a variety of 1,3-dienes have been well characterized.[16]

As can be seen in Scheme II, we envisioned the conversion of the biscarboethoxy group of cycloadduct **13** to the lactone carbonyl of **15** to proceed by either lead tetraacetate mediated oxidative decarboxylation[17] of diacid **14** or by the classical Curtius degradation[18] of the same intermediate.

Although a number of syntheses of δ,γ-unsaturated valerolactones are known,[18] one in particular is capable of generating only dialkyl-substituted species such as **15e** in high yield (eq 6). This method involves the addition of ketene to γ-alkyl-α,β-unsaturated ketones.[19a]



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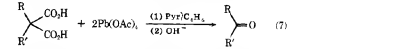
Diels–Alder Adducts of Diethyl Ketomalonate (12). The 4 + 2 cycloaddition products were made by dissolving **12** and an excess of the 1,3-diene in acetonitrile and heating the solution at 130–135 °C for the designated period of time in a sealed tube (Table III). The diesters **13a–f** were then hydrolyzed to the biscarboxylic acids **14a–f** in good overall yield based on diethyl ketomalonate.

Conspicuously absent from Table III is the cyclopentadiene adduct. Cycloaddition of this normally reactive diene had been reportedly unsuccessful.[16a] We also attempted the cyclization of monomeric cyclopentadiene with **12** at various temperatures ranging from −20 to 135 °C, but we were unable to isolate the cycloadduct. Apparently, if the adduct is formed, thermodynamic instability results in facile cycloreversion. Anthracene also failed to form a product with the carbonyl substrate.

Lactone Carbonyl Release. Lead Tetraacetate Method. Alkyl-substituted malonic acids undergo oxidative decarboxylation to form aldehydes or ketones upon treatment with lead tetraacetate,[20] LTA (eq 7). When similarly applied to



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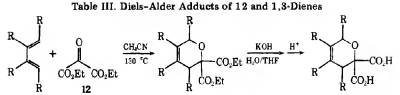
diacid **14c** (see Table IV), this procedure allowed isolation of the desired lactone in 20% yield after aqueous workup. Following numerous attempts to improve the yield of valerolactone **15c**, the best conditions were found to be a variation of a procedure developed by Cope and co-workers[21] utilizing sodium acetate to facilitate carboxyl ligand transfer to Pb^{IV}, a prerequisite for successful oxidation.[17] (Pyridine also functions in this manner.) However, due to limited success in generation of other valerolactones with lead tetraacetate, the Curtius degradation was explored.

Trimethylsilyl Azide Method. The essential feature of the Curtius degradation of carboxylic acids is the thermal rearrangement of an acyl azide to the isocyanate, and numerous approaches to the synthesis of acyl azides are known.[22] One procedure which has been developed recently is the one-pot conversion of an acid chloride to the isocyanate using trimethylsilyl azide (TMSA).[23] (See Scheme III.)

Upon treatment of a warm cyclohexane solution[24] of the bisacid chloride of **14c** with an excess of TMSA, the bisacyl azide was rapidly formed as evidenced by infrared spectroscopy (λ_{max} 4.67 and 5.80 μm). On further warming, stepwise rearrangement to the bisisocyanate apparently occurred. Within 15 min strong isocyanate infrared absorptions (λ_{max} 4.40 and 4.46 μm) were seen which were equal in intensity to those of the acyl azide. After about 30 min of heating, no acyl azide remained. Removal of solvent, followed by mild hydrolysis of a tetrahydrofuran solution of the bisisocyanate with either aqueous acetic acid or aqueous oxalic acid generated

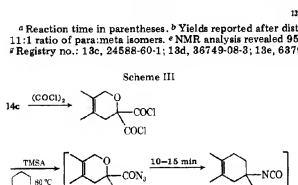
Table III. Diels–Alder Adducts of 12 and 1,3-Dienes



Registry no.	Diene[a]	Diester (%)[b,g]	Diacid (%)[c]	Mp, °C
	1,3-Butadiene (16 h)	 13a (78)	 14a (63)	Oil
	Isoprene (4 h)	 13b (80)[d]	 14b (64)	Oil
	2,3-Dimethyl-1,3-butadiene (4 h)	 13c (86)	 14c (70)	138–140
504-60-9	Piperylene (4 h)	 13d (85)[e]	 14d (66)	124–126
1118-58-7	2-Methyl-1,3-pentadiene (1.5 h)	 13e (95)[f]	 14e (90)	146–147
	1,3-Cyclohexadiene (4 h)	 13f (84)	 14f (74)	128–130

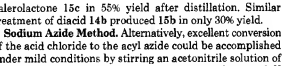
[a] Reaction time in parentheses. [b] Yields reported after distillation. [c] Overall yield based on 12. [d] NMR analysis revealed 11:1 ratio of para:meta isomers. [e] NMR analysis revealed 95% ortho isomer. [f] No meta isomer detected by NMR analysis. [g] Registry no.: 13c, 24588-60-1; 13d, 36749-08-3; 13e, 68797-64-8; 13f, 24588-62-3.

Scheme III



valerolactone 15c in 55% yield after distillation. Similar treatment of diacid 14b produced 15b in only 30% yield.

Sodium Azide Method. Alternatively, excellent conversion of the acid chloride to the acyl azide could be accomplished under mild conditions by stirring an acetonitrile solution of the acid chloride with an excess of activated sodium azide[25] at room temperature (eq 8). Within 40 min substitution was

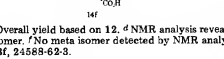
 (8)

complete and filtration of the reaction mixture to remove insoluble sodium salts, followed by thorough concentration at room temperature, generated the viscous product (λ_{max} 4.67 μm).

Without further purification, careful addition of cyclohexane to the potentially explosive acyl azide followed by rapid stirring at 80 °C for 40 min resulted in formation of the insoluble isocyanate. Hydrolysis of this material as before led to isolation of 15c in 72% yield (Table V) based on diacid. Extension of this method to other diacids resulted in improved yields of the desired valerolactones.

However, when the sodium azide method was applied to the bisacid chlorides of 14d and 14e, the dienoic acids 16d and 16e were recovered along with the lactone in the case of the latter material. Perhaps stabilization of an incipient cation by the allylic methyl group allows this rearrangement to take place during isocyanate hydrolysis (eq 9 and 10).

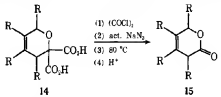
Table IV. LTA Oxidation of Diacids 14a-c



Diacid	Method	Lactone (%)[a]
14c	2 equiv of Pyr, C_6H_6, 80 °C, 3 h[b]	15c (20)
14c	NaOAc, HOAc, C_6H_6, 40 °C, 1 h[c]	15c (63)
14b	NaOAc, HOAc, C_6H_6, 40 °C, 1 h[c]	15b (40)
14a	NaOAc, HOAc, C_6H_6, 40 °C, 1 h[c]	15a (0)

[a] Yields reported after distillation. [b] Tufariello and Kissel, ref 20. [c] Cope, Park, and Scheiner, ref 21.

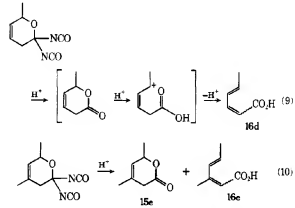
Table V. Lactone Formation by the Sodium Azide Method



14 15

Registry no.	Diacid	Hydrolysis method[a]	Time, min	Lactone (%)[b]	Other products (%)
57668-92-5	14a	A	40	15a (52)	.....
57668-93-6	14b	A	50	15b (60)	.....
57668-94-7	14c	A	60	15c (72)	.....
828-50-2	14d	B	60	.....	16d (55)
63797-65-9	14e	A	40	15e (30)	16e (34)
61779-36-0	14f	C		15f (7)	.....

[a] Method A: aqueous oxalic acid/THF/25 °C; method B: hydrolysis was run under a variety of conditions ranging from mildly acidic to mildly basic ($NaHCO_3$); method C: 5% aqueous $NaHCO_3$/THF/25 °C/18 h followed by acidification to pH 2. [b] Yields are reported after distillation and are based on diacid.



Synthetic Transformations of Lactone 15c. The synthetic versatility of these lactones is outlined in Scheme IV where lactone 15c was subjected to a variety of chemical manipulations.

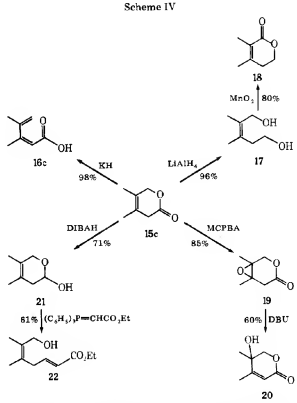
Production of 3,4-dimethyl-2(*Z*),4-pentadienoic acid (**16c**) was cleanly accomplished by treatment of the lactone with 1 equiv of potassium hydride in THF at 0 °C.

Lithium aluminum hydride reduction of the lactone in THF at room temperature afforded diol **17** in excellent yield. This product could be transformed to the α,β-unsaturated valerolactone **18** upon selective oxidation of the allylic alcohol using activated manganese dioxide.[26]

Epoxylactone **19** was prepared by oxidation of **15c** with *m*-chloroperbenzoic acid in dichloromethane at 0 °C. Treatment of the crystalline epoxide with diazabicyclo[5.4.0]-undec-5-ene (DBU) in THF generated the rearranged alcohol **20** in good yield.[27]

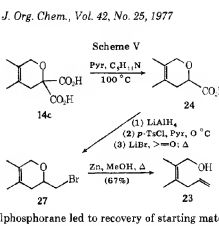
Reduction of **15c** to the cyclic hemiacetal **21** was achieved at −20 °C in ether using diisobutylaluminum hydride

Scheme IV

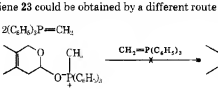


(DIBAH). Wittig condensation of the weakly basic stabilized ylide carboethoxyethylidene triphenylphosphorane with **21** generated the 1,4-diene **22** in 61% yield. Attempted Wittig reaction of **21** with the unstabilized ylide methylene tri-

Scheme V



phenylphosphorane led to recovery of starting material (eq 11). Diene **23** could be obtained by a different route starting



(11)

with the diacid **14c**. This compound could be monodecarboxylated using pyridine and a few equivalents of piperidine at 100 °C as shown in Scheme V. The new carboxylic acid **24** was then reduced to the alcohol **25** using LAH in ether. Conversion to the *p*-toluenesulfonate **26** was accomplished using standard procedures, and displacement by lithium bromide in refluxing acetone then generated the primary bromide in 70% overall yield from **25**. Zinc-mediated fragmentation of the bromide was performed in refluxing methanol to afford the desired product.

Experimental Section

Reactions were carried out under a nitrogen atmosphere unless otherwise noted. Melting points were taken on a Fisher-Johns hot stage apparatus and are uncorrected. IR spectra were determined on a Perkin-Elmer 137 spectrophotometer. NMR spectra were taken on either Varian T-60 or A-60A spectrometers with tetramethylsilane as an internal standard. In describing NMR chemical shifts, peaks are reported by indicating the center of the pattern. The multiplicity of the peak is abbreviated as s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. Mass spectra were obtained using a Hitachi Perkin-Elmer Model RMU-7E spectrometer. Elemental analyses were determined by Robertson Laboratory, Florham Park, N.J. Micro thin-layer chromatography was performed on Eastman Chromatogram Sheets No. 960 precoated with silica gel and fluorescent indicator. Preparative thick-layer chromatography was done on precoated Silica Gel G-200 plates with fluorescent indicator as supplied by Brinkman Instruments, Inc. Column chromatography was conducted with Grace silica gel, grade 923, 100–200 mesh.

All chemicals were commercial samples unless reference is given to their preparation. They were used as received unless otherwise noted. Anhydrous solvents were obtained by distillation from the specified substances: acetonitrile, chloroform, and dichloromethane from P_2O_5; benzene and methanol from calcium hydride; cyclohexane from sulfuric acid; ether and tetrahydrofuran (THF) from sodium benzophenone ketyl.

General Procedure for Cycloaddition of Vinyltriphenylphosphonium Bromide with 1,3-Dienes. A solution of an excess of freshly distilled 1,3-butadiene (precooled to −78 °C), vinyltriphenylphosphonium bromide (2.50 g, 7 mmol), and a trace of hydroquinone in 5 mL of acetonitrile was heated in a sealed tube at 145 °C. After 20 h the tube was opened and the contents were removed with CH_2Cl_2. Following concentration at reduced pressure, the gummy residue was dissolved in a minimal volume of $CHCl_3$ and triturated with Et_2O to afford, after drying under vacuum, 2.90 g (93%) of cyclohex-3-enyltriphenylphosphonium bromide (**3a**): mp 240–241 °C dec; NMR ($CDCl_3$) δ 5.70 (br s, 2 H), 5.00–5.50 (br m, 1 H), 1.90–3.00 (complex m, 6 H). Anal. Calcd for $C_{24}H_{24}BrP$: C, 68.09; H, 5.71. Found: C, 67.66; H, 5.26.

In a similar fashion, the following phosphonium salts were prepared.

Table VI. Spectral and Analytical Data

Compound	NMR (δ)[c]	Analysis,[b] mol wt
5a	5.70–6.0 (m, 3 H)	Calcd: 170.2588 Found: 170.2610
5b	6.15 (br s, 1 H), 5.70 (br s, 1 H), 1.75 (br s, 3 H)	Calcd: 183.2796 Found: 183.2808
5c	6.20 (br s, 1 H), 1.70 (br s, 6 H)	Calcd: 198.1401 Found: 198.1392
5d	6.30 (br s, 1 H), 6.02 (ABq, J = 2 Hz, 2 H)	Calcd: 182.1093 Found: 182.1102
5e	6.10–6.25 (m, 3 H), 3.09 (br s, 1 H), 2.71 (br s, 1 H)	Calcd: 196.1262 Found: 196.1279
7	6.20–6.35 (m, J = 3.5 Hz, 2 H), 4.55, 4.70 (2m, 2 H)	Calcd: 120.1963 Found: 120.1982
8	6.24 (m, J = 4 Hz, 2 H), 5.15 (br t, 1 H)	Calcd: 204.3571 Found: 204.3558
9	6.25 (m, J = 4 Hz, 2 H), 5.13–6.05 (m, 3 H), 1.75 (d, J = 6 Hz, 3 H)	Calcd: 160.2689 Found: 160.2702
10	6.40 (q, 2 H), 3.02 (br m, 2 H), 1.95 (d, J = 3 Hz, 2 H), 0.95–1.85 (complex m, 2 H)	Calcd: 122.0731 Found: 122.0720

[a] Spectra taken in CCl_4. [b] By mass spectral analysis.

4-Methylcyclohex-3-enyltriphenylphosphonium bromide (3b): mp 232–235 °C dec; NMR ($CDCl_3$) δ 5.30–5.60 (br s, 1 H), 4.40–4.95 (br m, 1 H), 2.00–3.00 (br m, 5 H), 1.65 (br s, 3 H). Anal. Calcd for $C_{25}H_{26}BrP$: C, 68.65; H, 5.99. Found: C, 68.04; H, 5.16.

3,4-Dimethylcyclohex-3-enyltriphenylphosphonium bromide (3c): mp 114–115 °C dec; NMR ($CDCl_3$) δ 6.23 (s, 1 H), 2.90 (br s, 2 H), 1.90–2.56 (br m, 4 H), 1.55 (s, 6 H). anal. Calcd for $C_{26}H_{28}BrP$: C, 69.18; H, 6.25. Found: C, 70.04; H, 5.98.

Bicyclo[2.2.1]hept-5-enyl-2-triphenylphosphonium bromide (3d): mp 220–223 °C dec; NMR ($CDCl_3$) δ 5.10–5.95 (br m, 3 H). Anal. Calcd for $C_{25}H_{24}BrP$: C, 68.97; H, 5.55. Found: C, 69.10; H, 5.93.

Bicyclo[2.2.2]oct-5-enyltriphenylphosphonium bromide (3e): mp 263–266 °C; NMR ($CDCl_3$) δ 5.10–6.03 (br m, 3 H). Anal. Calcd for $C_{26}H_{26}BrP$: C, 69.49; H, 5.83. Found: C, 70.05; H, 6.18.

General Procedure for Wittig Condensation. To a cooled solution (0 °C) of the phosphorane of **3e** (0.442 g, 1 mmol) in 10 mL of THF (prepared by addition of 1.1 mmol of lithium diisopropylamide in THF to a suspension of phosphonium salt in the same solvent at −78 °C) was added 0.127 g (1.2 mmol) of benzaldehyde. After stirring overnight at 25 °C, the product was diluted with pentane and the organic layer was washed many times with water, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The pure olefin was obtained by column chromatographic purification over silica gel using hexane as eluent. Thus, 2-benzylidenebicyclo[2.2.2]oct-5-ene (**5e**) was obtained (0.166 g, 85%) as a colorless oil. Anal. Calcd for $C_{15}H_{16}$: mol wt, 196.1279. Found: mol wt (MS), 196.1262.

Table VI contains spectral and analytical data for Wittig products **5a–d, 7, 8, 9,** and **10.**

Bicyclo[2.2.2]oct-5-en-2-one (10). Phosphorane **3e** (0.442 g, 1 mmol) was prepared as above. Oxygen was bubbled into the stirring suspension until the characteristic deep red color of the ylide disappeared. After 24 h, the resulting mixture was directly filtered through silica gel to afford 0.024 g (20%) of ketone **10**: IR (hexane) 5.78, 6.2 μm.

Bicyclic Aldehyde (6). To a cooled solution (0 °C) of the ylide of phosphonium salt **3d** (1.254 g, 3 mmol) in 50 mL of THF was added glyoxal monodiethyl acetal[9] (0.53 g, 4 mmol). After stirring overnight, the product was diluted with pentane and the organic layer was repeatedly washed with water and dried over $MgSO_4$. The crude diethyl acetal was obtained by removal of solvent by distillation at atmospheric pressure. The volatile product was redissolved in pentane and stirred overnight with 3 g of silica gel at room temperature. After filtration and concentration at atmospheric pressure, the crude aldehyde was purified by thick-layer chromatography using 4:1 hexane–ether

as developing solvent and obtained as a colorless volatile liquid (0.14 g, 39%, a mixture of *Z* and *E* isomers): IR (CH_2Cl_2) 5.97, 6.07, and 6.17 μm; NMR ($CDCl_3$) δ 9.80 (d, J = 8.5 Hz, *CHO*, *Z* isomer); 9.60 (d, J = 8.5 Hz, *CHO*, *E* isomer), 4.22 (m, doubly allylic methine *Z* isomer), 3.42 (m, doubly allylic methine *E* isomer); mp (semicarbazide) 207–208 °C dec. Anal. Calcd for $C_{10}H_{13}N_3O$: mol wt, 191.2258. Found: mol wt, 191.2284.

General Procedure for Cycloaddition of Diethyl Ketomalonate with 1,3-Dienes. Freshly distilled 1,3-butadiene (an excess) was condensed into a Carius tube containing a trace of hydroquinone, diethyl ketomalonate (1.82 mL, 12 mmol) and 4 mL of acetonitrile. The tube was sealed and after heating at 135 °C for 16 h, the product was isolated with CH_2Cl_2. After concentration, approximately 50 mL of 95% EtOH was added to precipitate polymeric material and the resulting white suspension was vacuum-filtered through Celite and then concentrated. Evaporative distillation produced diethyl 3,6-dihydropyran-2,2-biscarboxylate (13a) (2.17 g, 79%) as a colorless liquid; bp 100 °C (0.8 mm); IR (neat) 5.71 μm; NMR (CCl_4) δ 5.79 (s, 2 H), 4.23 (q and buried s, 6 H), 2.65 (m, 2 H) and 1.27 (t, 6 H). Anal. Calcd for $C_{13}H_{20}O_5$: mol wt, 256.1317. Found: mol wt, 256.1320.

In a similar fashion the following known compounds were prepared.

Diethyl 4-Methyl-3,6-dihydropyran-2,2-bisarboxylate (13b). Isoprene (2.7 mL, 27 mmol) and diethyl ketomalonate (1.82 mL, 12 mmol) were treated as above. After 4 h of heating, identical workup afforded 13b as a colorless liquid after distillation (2.4 g, 80%). Faramete isomer ratio was 11:1 as determined by NMR analysis; bp 110 °C (1.0 mm); IR (neat) 5.70 μm; NMR (CCl_4) δ 5.38 (s, 1 H), 4.23 (q and buried s, 6 H), 2.50 (s, 2 H), 1.76 (s, *p*-CH_3), 1.65 (s, *m*-CH_3).

Diethyl 4,5-Dimethyl-3,6-dihydropyran-2,2-bisarboxylate (13c). 2,3-Dimethyl-1,3-butadiene (2.7 mL, 25 mmol) and diethyl ketomalonate (1.82 mL, 12 mmol) were treated as above. After 4 h heating, similar workup afforded 13c (2.64 g, 86%); bp 120 °C (1.0 mm); IR (neat) 5.72 μm; NMR (CCl_4) δ 4.20 (q and buried s), 2.46 (s, 2 H), 1.69 and 1.52 (2s, 6 H).

Diethyl 6-Methyl-3,6-dihydropyran-2,2-bisarboxylate (13d). Piperylene (2.7 mL, 27 mmol) and diethyl ketomalonate (1.55 mL, 10.2 mmol) were treated as above to afford 13d (2.19 g, 85%); bp 105 °C (1.5 mm); IR (neat) 5.73 μm; NMR (CCl_4) δ 5.62 (m, 2 H), 4.20 (q and buried m, 5 H), 2.58 (m, 2 H) and 1.30 (m, 9 H). No 3-methyl isomer was detected by NMR analysis.

Diethyl 4,6-Dimethyl-3,6-dihydropyran-2,2-bisarboxylate (13e). 2-Methyl-1,3-pentadiene (4 mL, 40 mmol) and diethyl ketomalonate (2.7 mL, 18 mmol) were treated as above to produce, after 90 min heating, adduct 13e (4.50 g, 95%): bp 112 °C (1.0 mm); IR (neat) 5.73 μm; NMR (CCl_4) δ 5.38 (m, 1 H), 4.33 (q and buried m, 5 H), 2.58 (m, 2 H), 1.78 (s, 3 H). No 4,6-dimethyl isomer was detected by NMR analysis.

2-Oxa-3,3-dicarboethoxybicyclo[2.2.2]oct-5-ene (13f). 1,3-Cyclohexadiene (2.7 mL, 27 mmol) and diethyl ketomalonate (1.82 mL, 12 mmol) were treated as above. After 4 h heating, similar workup afforded 13f (2.86 g, 84%): bp 120 °C (0.8 mm); IR (neat) 5.72 μm; NMR (CCl_4) δ 6.45 (m, 2 H), 4.50 (br m, 1 H), 4.15 (m, 4 H), 3.30 (br m, 1 H), 1.6–2.5 (complex m, 4 H).

General Procedure for Hydrolysis of Diester 13 to Diacid 14. To a solution of diester 13a (0.821 g, 3.4 mmol) in 30 mL of THF was added 30 mL of 10 N KOH and the resulting mixture was stirred at room temperature for 30 h. Acidification to pH 1 with 2 N HCl was followed by thorough extraction with Et_2O and the combined organic extracts were dried over Na_2SO_4. Complete removal of solvent afforded 3,6-dihydropyran-2,2-bischaroxylic acid (14a) as a golden viscous oil (0.50 g, 80%) (dry by NMR analysis) which resisted numerous crystallization attempts: IR (Et_2O) 5.78 μm; NMR ($CDCl_3$) δ 5.78 (s, 2 H), 4.42 (s, 2 H) and 2.78 (s, 2 H).

4-Methyl-3,6-dihydropyran-2,2-bisarboxylic Acid (14b). Diester 13b (0.988 g, 4 mmol) was treated as above. The resulting diacid 14b was also obtained as a golden viscous oil (0.670 g, 85%): IR (neat) 5.75 μm; NMR ($CDCl_3$) δ 5.57 (s, 1 H), 4.53 (s, 2 H), 2.78 (s, 2 H), 1.90 (s, *p*-vinyl CH_3) and 1.88 (*m*-vinyl CH_3).

4,5-Dimethyl-3,6-dihydropyran-2,2-bisarboxylic Acid (14c). Diester 13c (2.640 g, 10.5 mmol) was treated as above. Diacid 14c was isolated as crystals and recrystallization from Et_2O–petroleum ether afforded 1.80 g (87%) of product: IR (CH_2Cl_2) 5.75 μm; NMR ($CDCl_3$) δ 4.25 (s, 2 H), 2.63 (s, 2 H) and 1.70, 1.55 (2s, 6 H).

6-Methyl-3,6-dihydropyran-2,2-bisarboxylic Acid (14d). Diester 13d (2.187 g, 9.5 mmol) was treated as above. Diacid 14d was obtained as a powdery solid after recrystallization (1.38 g, 78%): NMR ($CDCl_3$) δ 5.75 (m, 2 H), 4.67 (br m, 1 H), 2.72 (ABq, J_{AB} = 15 Hz, 2 H) and 1.33 (d, J = 6 Hz, 3 H).

4,5-Dimethyl-3,6-dihydropyran-2,2-bisarboxylic Acid (14e). Diester 13e (4.50 g, 16.9 mmol) was treated as above to afford diacid 14e (3.24 g, 90%) as a powder: NMR ($CDCl_3$) δ 5.35 (m, 1 H), 4.58 (br m, 1 H), 2.85 (ABq, J_{AB} = 18 Hz, 2 H), 1.78 (s, 3 H) and 1.30 (d, J = 6 Hz, 3 H).

2-Oxa-3,3-dicarboxybicylo[2.2.2]oct-5-ene (14f). Diester 13f (2.50 g, 8.8 mmol) was treated as above to generate diacid 14f (1.78 g, 90%) as a powder: IR (CH_2Cl_2) 5.68 μm; NMR ($CDCl_3$) δ 6.10 (complex m, 2 H), 5.17 (br m, 1 H), 2.7 (br m, 1 H).

Lactone Carbonyl Release—Representative Procedures. 3,4-Dimethyl-3,4-dehydrovalerolactone (15c). Lead Tetraacetate Oxidation. Lead tetraacetate (1.00 g, 2 mmol, 90% in acetic acid) was added to a flask containing a suspension of excess anhydrous $NaOAc$ in 2 mL of dry benzene and 1 mL of glacial HOAc. The mixture was then heated to 65 °C. Diacid 14c (0.10 g, 0.5 mmol) was dissolved in 2 mL of glacial HOAc and then introduced into the preheated mixture. Immediate CO_2 evolution was observed and the oxidation was allowed to proceed for 1 h.

Upon cooling, the resulting white suspension was extracted with Et_2O and washed with water, neutralized with aqueous $NaHCO_3$, washed with brine, and dried over $MgSO_4$. After concentration and Kugelrohr distillation of the residue, lactone 15c was recovered in 63% yield (0.040 g, yield based on diacid): bp 105 °C (1.0 mm); IR (neat) 5.75, 5.98 μm; NMR (CCl_4) δ 4.60 (s, 2 H), 2.85 (s, 2 H) and 1.70 (s, 6 H); *m/e* 126, 110, 108, 82, 69, 67, 55, 54, 53. Anal. Calcd for $C_7H_{10}O_2$: mol wt, 126.0681. Found: mol wt, 126.0667.

Trimethylsilyl Azide Method. To a stirring suspension of diacid 14c (0.1 g, 0.5 mmol) in 25 mL of benzene containing a catalytic amount of pyridine was added 0.254 g (2 mmol) of oxalyl chloride. The reaction mixture was heated at reflux temperature until formation of acid chloride was complete, i.e., 2 h (IR, 5.59 μm). After removal of solvent and traces of oxalyl chloride, the acid chloride was dissolved in cyclohexane and heated to reflux. A solution of trimethylsilyl azide (0.180 g, 1.5 mmol) in 5 mL of cyclohexane was then added. Isocyanate formation was accomplished in about 40–50 min (IR, 4.40 and 4.46 μm).

After the product was allowed to cool, 10 mL of a 2:1 HOAc–H_2O solution was added and the mixture was stirred for 1 h at 25 °C. Following thorough Et_2O extraction, the combined organic portions were washed with water, neutralized with aqueous $NaHCO_3$, washed with brine, and dried over $MgSO_4$. Concentration afforded lactone 15c in 57% yield (0.035 g, based on diacid).

Sodium Azide Method. (These bisacyl azides are potentially explosive and should be handled behind a safety shield.) The bisacid chloride of diacid 14c (0.189 g, 0.95 mmol) was prepared as above using 0.30 g (2.3 mmol) of oxalyl chloride. After isolation of the crude product, the resulting oil was redissolved in dry acetonitrile (25 mL) and activated sodium azide[25] (0.25 g, 3.8 mmol) was added. The suspension was stirred at room temperature for 45 min (IR 4.67, 5.81, and 5.85 μm).

The reaction mixture was quickly filtered and then concentrated at room temperature under reduced pressure to afford a gummy golden residue of the bisacyl azide. (CAUTION! The bisacyl azide is shock sensitive. Cover with solvent before introduction of magnetic stirring bar.)

Curtius rearrangement was effected as before by vigorously stirring the insoluble residue in dry cyclohexane at reflux temperature. Formation of the insoluble bisisocyanate normally required 45–50 min (IR 4.40 and 4.46 μm). After concentration and redissolution in THF, hydrolysis was accomplished using 3 mL of 5% squeous oxalic acid and stirring at 25 °C for 1 h.

Isolation of lactone 15c was performed according to the same procedure as above generating 0.086 g (72%, based on diacid) of the desired compound.

3,4-Dehydrovalerolactone (15a). The sodium azide method was employed as above. Diacid 14a (0.5 g, 2.8 mmol) afforded 0.120 g (52%) of the desired lactone 15a. Hydrolysis time was shortened to 40 min: bp 97–100 °C (0.8 mm); IR (neat) 5.69 μm; NMR (CCl_4) δ 5.88 (s, 2 H), 4.83 (m, 2 H), and 2.98 (m, 2 H); *m/e* 98, 70, 54, 43, 39. Anal. Calcd for $C_5H_6O_2$: mol wt, 98.0368. Found: mol wt, 98.0404.

3-Methyl-3,4-dehydrovalerolactone (15b). The sodium azide method was employed as above. Diacid 14b (0.130 g, 0.7 mmol) generated 0.042 g, (60%) of the desired lactone 15b. Hydrolysis time was 50 min: bp 100–102 °C (1.0 mm); IR (neat) 5.76 μm; NMR (CCl_4) δ 5.60 (s, 1 H), 4.79 (s, 2 H), 2.91 (s, 2 H) and 1.80 (s, 3 H); *m/e* 112, 84, 82, 69, 55, 41. Anal. Calcd for $C_6H_8O_2$: mol wt, 112.0524. Found: mol wt, 112.0518.

3,5-Dimethyl-3,4-dehydrovalerolactone. (15e). The sodium azide method was employed as above. Diacid 14e (0.2 g, 1 mmol) afforded 0.034 g (30%) of the desired lactone 15e. Hydrolysis time was

45 min: bp 98–101 °C (1.0 mm); IR (neat) 5.74 μm; NMR (CCl_4) δ 5.58 (m, 1 H), 5.10 (m, 1 H), 2.98 (m, 2 H), 1.80 (s, 3 H) and 1.45 (d, 3 H); *m/e* 126, 110, 98, 83, 67, 55, 43. Anal. Calcd for $C_7H_{10}O_2$: mol wt, 126.0681. Found: mol wt, 126.0674.

3-Methylhexa-2,4-dienoic Acid (16e). Upon concentration of the crude parent lactone **15e** during the isolation procedure above, a solid appeared. Following addition of 5 mL of Et_2O, the supernatant containing the lactone was removed. Recrystallization of the solid from $CHCl_3$–Et_2O produced the dienoic acid **16e** (0.036 g, 34%): mp 119–121 °C; IR (CH_2Cl_2) 5.87 μm; NMR ($CDCl_3$) δ 7.58 (m, 1 H),[28] 6.60–5.46 (m, 3 H, vinyl H and OH) and 1.97 (m, 6 H). Anal. Calcd for $C_7H_9O_2$: (M − 1)*e*, 125.0602. Found: (M − 1)*e*, 125.0586.

Hexa-2,4-dienoic Acid (16d). The sodium azide method was employed as above using diacid **14d** (0.186 g, 1 mmol). Following hydrolysis, extraction and isolation afforded the dienoic acid **16d**, presumably a mixture of isomers, after recrystallization from $CHCl_3$–Et_2O (0.061 g, 55%). No lactone precursor was found: mp 115–117 °C; NMR ($CDCl_3$) δ 7.43 (m, 1H),[28] 6.60–5.50 (complex m, 4 H, vinyl H and OH), and 1.82 (d, 3 H); *m/e* 112, 111, 92, 67, 41, 29. Anal. Calcd for $C_6H_8O_2$: mol wt, 112.0524. Found: mol wt, 112.0514.

Bicyclic Lactone (18*f*). The sodium azide method was used as shown above. Diacid **14*f*** (0.31 g, 1.7 mmol) afforded the bisacyl chloride (5.58 μm) after 24 h at reflux, then the bisacyl azide (4.63 and 5.81 μm) and the bisisocyanate (4.39 and 4.43 μm). Lactone carbonyl release was effected by dissolution of the bisisocyanate in 20 mL of THF followed by addition of 10 mL of 5% aqueous $NaHCO_3$. After stirring at 25 °C overnight, the basic mixture was acidified to pH 2 and thoroughly extracted into Et_2O. Drying (Na_2SO_4) and concentration led to recovery of a golden oil. Evaporative distillation afforded the lactone **15*f*** as a colorless liquid (0.015 g, 7% from diacid): IR (neat) 5.71, 6.20, 11.46 μm; NMR (CCl_4) δ 6.50 (m, 2 H), 5.10 (br m, 1 H), 3.40 (br m, 1 H) and 1.35–2.30 (complex m, 4 H); *m/e* 124, 96, 80, 79, 78, 77, 68. Anal. Calcd for $C_7H_8O_2$: mol wt, 124.0524. Found: mol wt, 112.0558.

***cis*-2,3-Dimethylpent-2-ene-1,5-diol (17).** To a suspension of lithium aluminum hydride (0.046 g, 1.2 mmol) in 40 mL of dry THF at 25 °C was slowly added a THF solution of lactone **15c** (0.126 g, 1 mmol). The reduction was allowed to proceed overnight. After addition of 2 mL of 10% aqueous NaOH, followed by stirring for 10 min, the contents were repeatedly extracted into Et_2O. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to afford diol **17** (0.114 g, 98%) as a colorless oil which needed no further purification: IR (neat) 3.0 and 6.04 μm; NMR (CCl_4) δ 4.47 (s, 2 H), 3.93 (s, 1 H), 3.57 (t, 2 H), 2.29 (t, 2 H), 1.76 and 1.70 (2s, 6 H); *m/e* 130, 112, 97, 84, 82, 67, 55, 55. Anal. Calcd for $C_7H_{14}O_2$: mol wt, 130.0994. Found: mol wt, 130.0982.

2,3-Dimethyl-2,3-dehydrovalerolactone (18). A solution of diol **17** (0.038 g, 0.29 mmol) in 6 mL of CH_2Cl_2 was stirred at room temperature. Excess activated manganese dioxide (0.35 g, 4 mmol) was slowly added and the mixture was stirred overnight. Isolation of lactone **18** was accomplished by dilution of the suspension with 10 mL of CH_2Cl_2 and then filtration through Celite. After removal of solvent, the product was obtained in 80% yield (0.029 g): IR (neat) 5.83 μm; NMR (CCl_4) δ 4.27 (t, 2 H), 2.58 (t, 2 H), 1.96 and 1.86 (2s, 6 H); *m/e* 126, 96, 81, 68, 67, 55, 43, 41. Anal. Calcd for $C_7H_{10}O_2$: mol wt, 126.0681. Found: mol wt, 126.0678.

3,4-Dimethyl-2(*Z*),4-pentadienoic Acid (16*c*). Potassium hydride (0.03 g, 1 mmol, 24% in oil) was washed three times with petroleum ether and then suspended in 10 mL of THF. A solution of lactone **15c** (0.063 g, 0.5 mmol) was added to the stirring mixture with immediate evolution of H_2 accompanied by formation of a pale yellow solid. Following acidification with dilute HCl, the contents were extracted with Et_2O and the organic layer was washed with water and brine and then dried over Na_2SO_4. After removal of solvent, feathery white crystals were isolated. Recrystallization from $CHCl_3$–Et_2O afforded the *cis*-dienoic acid **16c** in 98% yield (0.062 g): mp 54.5–56 °C; IR (CH_2Cl_2) δ 89, 6.05, and 6.08 μm; NMR ($CDCl_3$) δ 5.68 (m, 1 H), 4.88 (m, 1 H), 4.70 (m, 1 H), and 1.96 (m, 6 H); *m/e* 126, 125, 111, 79, 55, 53. Anal. Calcd for $C_7H_{10}O_2$: mol wt, 126.0681. Found: mol wt, 126.0677.

3,4-Dimethyl-3,4-epoxyvalerolactone (19). A solution of lactone **15c** (0.126 g, 1 mmol) in 20 mL of CH_2Cl_2 was cooled to 0 °C; 100% *m*-chloroperbenzoic acid[29] (0.344 g, 2 mmol) was then added as a solution in CH_2Cl_2. After 4 h, excess oxidizing agent was destroyed with 10 mL of saturated $NaHSO_3$ solution. Isolation was accomplished with CH_2Cl_2 extraction. After neutralization of the organic extract with aqueous $NaHCO_3$ and drying over $MgSO_4$, the solvent was removed to afford white needle crystals (0.120 g, 85%) of epoxide **19**: mp 49–51 °C; NMR (CCl_4) δ 4.28 (ABq, J_{AB} = 12 Hz, 2 H), 2.76 (s, 2 H) and 1.22 (s, 6 H); *m/e* 112, 99, 83, 69, 43. Anal. Calcd for $C_6H_8O_2$ (M − 30): 112.0524. Found: 112.0532. Loss of CH_2O produces the major fragment.

3,4-Dimethyl-4-hydroxy-2-dehydrovalerolactone (20). Epoxide **19** (0.044 g, 0.35 mmol) was dissolved in 10 mL of THF. 1,5-Diazabicyclo[5.4.0]undec-5-ene (DBU) (0.053 g, 0.35 mmol) was introduced into the solution and the reaction was allowed to proceed at 25 °C for 3 h. Dilute HCl (6 mL) was added and the contents of the flask were extracted into CH_2Cl_2. After the organic layer was washed with water and dried over Na_2SO_4, concentration led to recovery of 0.032 g (60%) of hydroxylactone **20** as a colorless liquid: IR (neat) 2.98 and 5.80 μm; NMR ($CDCl_3$) δ 5.74 (m, J_{AX} = 0.7 Hz, 1 H, vinyl H), 4.17 (s, 2 H), 2.74 (br s, 1 H), 2.01 (d, J_{AX} = 0.7 Hz, 3 H, vinyl CH_3) and 1.37 (s, 3 H). Anal. Calcd for $C_7H_{10}O_3$: mol wt, 142.1625. Found: mol wt, 142.1514.

3,4-Dimethyl-3,4-dehydrovalerolactol (21). Lactone **15c** (0.252 g, 2 mmol) was dissolved in 10 mL of dry Et_2O and cooled to −20 °C. Diisobutylaluminum hydride (4 mL, 5.2 mmol, 20% in hexane) was slowly added by syringe to the precooled solution and after 30 min, 2 mL of MeOH was introduced. The mixture was stirred overnight and, after dilution with Et_2O, the organic layer was washed with brine, dried over $MgSO_4$, and filtered through Celite. Concentration led to recovery of the crude product. Purification was accomplished by column chromatographic separation over silica gel. Lactol **21** was eluted with Et_2O and obtained as a colorless liquid (0.18 g, 71%): IR (neat) 2.90 μm; NMR ($CDCl_3$) δ 5.01 (t, 1 H), 3.99 (br s, 2 H), 2.10 (br s, 3 H) and 1.58 (d, 6 H); *m/e* 110, 95, 82, 77, 64, 51, 41. Anal. Calcd for $C_7H_{10}O$ (M − 18), 110.0732. Found: 110.0737. Loss of H_2O produces the major fragment.

Ethyl 5,6-Dimethyl-7-hydroxyhepta-2 (*E*),5(*Z*)-dienoate (22). Lactol **21** (0.064 g, 0.5 mmol) was dissolved in 15 mL of benzene contained in a 25-mL two-neck flask fitted with a reflux condenser. A benzene solution of carboethoxyethylidene triphenylphosphorane was then added to the reaction flask and heated to reflux. After 20 h, the mixture was cooled and then diluted with H_2O. Following extraction with Et_2O and drying over Na_2SO_4, the organic extract was concentrated to furnish the crude product. Preparative thick layer chromatography on silica gel afforded, after development with 1:1 hexane–Et_2O, diene **22** as a colorless liquid (0.058 g, 61%); IR (neat) 2.90, 5.80 and 5.85 μm; NMR ($CDCl_3$) δ 6.95 (d of t, J_{AB} = 14 Hz, J_{AC} = 7 Hz, 1 H), 5.80 (d, 1 H), 3.0 (d, 2 H), 4.20 (m, 5 H), 1.75 (d, 6 H), 1.22 (t, 3 H). Anal. Calcd for $C_{11}H_{18}O_3$: mol wt, 198.2620. Found: mol wt, 198.2603.

Production of Monocarboxylic Acid 24. A solution of diacid **14c** (1.40 g, 7.0 mmol) in 40 mL of dry pyridine containing 1 mL of piperidine was heated at 100 °C for 5 h. After the reaction mixture was allowed to cool, it was diluted with Et_2O and thoroughly extracted with dilute HCl to remove traces of base. The remaining Et_2O layer was washed with H_2O, then with brine, and dried over Na_2SO_4. Removal of solvent led to recovery of the monocarboxylic acid **24** as white crystals (0.940 g, 88%): mp 84.5–85 °C; IR (CH_2Cl_2) 5.72 μm; NMR ($CDCl_3$) δ 4.27 (partially buried t, 1 H), 4.13 (br s, 2 H), 2.30 (br d, 2 H), 1.68 and 1.53 (2s, 6 H); *m/e* 156, 138, 111, 110, 109, 96, 95, 83, 67, 55. Anal. Calcd for $C_8H_{12}O_3$: mol wt, 156.0786. Found: mol wt, 156.0512.

LAH Reduction of 24. LAH (0.5 g, 5 mmol) was suspended in Et_2O and the mixture was stirred at 0 °C for 20 min. An ethereal solution of carboxylic acid **24** (1.1 g, 5.5 mmol) was added dropwise to the hydride suspension and the mixture was then stirred for 2 h at 25 °C. Excess hydride was quenched by cautious addition of H_2O, followed by 10 mL of 0.1 N NaOH. After stirring for 30 min, the contents were extracted with Et_2O. The organic layer was washed with H_2O, then with brine, and dried over $MgSO_4$. Concentration and evaporative distillation led to recovery of 0.58 g (74%) of alcohol **25** as a colorless liquid: bp 105 °C (2 mm); IR (neat) 2.90 μm; NMR (CCl_4) δ 3.94 (br s, 2 H), 3.48 (br s and buried m, 4 H), 1.8–1.96 (br m, 2 H), 1.54 and 1.65 (2s, 6 H). Anal. Calcd for $C_8H_{14}O_2$: mol wt, 142.0998. Found: mol wt, 142.1013.

Preparation of Tosylate 26 and Bromide 27. To a solution of alcohol **25** (0.46 g, 3.2 mmol) in 10 mL of pyridine cooled to 0 °C was added 1.25 g (6.5 mmol) of *p*-toluenesulfonyl chloride. After 8 h at 0 °C, ice chips were added to destroy excess *p*-tosyl chloride, and the product was isolated with Et_2O. Yield of crude tosylate **26**, 0.81 g (83%); IR (CH_2Cl_2) 8.39 and 8.47 μm; NMR (CCl_4) δ 7.23 and 7.67 (centers of 2d of ABq, J_{AB} = 8 Hz, 4 H), 2.40 (s, 3 H).

The crude tosylate (0.81 g, 2.74 mmol) was then dissolved in 30 mL of anhydrous acetone. Lithium bromide (0.952 g, 11 mmol) was added and the solution was heated at reflux for 20 h. The solution was allowed to cool and, after removal of acetone, the residue was extracted with Et_2O. After drying over Na_2SO_4, the organic extract was concentrated to a brown liquid. Filtration through silica gel and Celite

afforded bromide **27** (0.475 g, 85%): IR (CCl_4) 7.98 and 8.08 μm; NMR (CCl_4) δ 3.95 (br s, 2 H), 3.61 (m, 1 H), 3.20–3.30 (m, 2 H), 2.0 (br m, 2 H), and 1.62 and 1.50 (2s, 6 H).

1-Hydroxy-2,3-dimethylbicyclo[2.2.1]hept-2(Z),5-diene (23). Bromide **27** (0.40 g, 1.94 mmol) was dissolved in 25 mL of dry methanol. Activated zinc (2.80 g, prepared by stirring zinc dust for 5 min in glacial HOAc and then washing with several portions of methanol) was then added to the solution and stirred at reflux temperature for 20 h. After the reaction mixture had cooled, it was filtered through Celite to remove the zinc. The product (0.160 g, 67%) was obtained after Et_2O extraction and distillation (bp 92 °C (2 mm)) using a Kugelrohr apparatus: IR (CH_2Cl_2) 2.89, 6.00, and 6.10 μm; NMR (CCl_4) δ 5.37–6.03, 5.0, and 4.79 (3 m, 5 H), 3.96 (s, 1 H), 3.42 (s, 1 H), 2.80 (d, J = 6 Hz, 2 H) and 1.63 and 1.70 (2d, 6 H). Anal. Calcd for $C_9H_{14}O$: mol wt, 126.1045. Found: mol wt, 126.1034.

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Registry No.—*exo*-3d, 63797-66-0; *endo*-3d, 63797-67-1; *exo*-3e, 63864-73-3; *endo*-3e, 63864-74-4; 4, 5044-52-0; (*E*)-5a, 63797-68-2; (*Z*)-5a, 63797-69-3; (*E*)-5b, 63797-70-6; (*Z*)-5b, 63797-71-7; (*E*)-5c, 63797-72-8; (*Z*)-5c, 63797-73-9; (*E*)-5d, 28764-49-0; (*Z*)-5d, 28764-48-9; (*E*)-5e, 63797-74-0; (*Z*)-5e, 63797-75-1; (*E*)-6, 63797-76-2; (*Z*)-6, 63797-77-3; 6 semicarbazide, 63797-78-4; 7, 19386-05-1; (*E*)-8, 63797-79-5; (*Z*)-8, 63797-80-8; 9, 54222-72-9; 10, 2220-40-8; 12, 606-09-6; 13a, 24688-58-7; 13b, 24688-59-8; 15a, 28677-08-7; 15b, 10021-22-4; 15c, 22937-02-6; 15e, 22936-96-5; 16c, 63797-81-9; (*E*)-16d, 110-44-1; (*Z*)-16d, 5309-57-9; (*E*)-16e, 63797-82-0; (*Z*)-16e, 28050-06-6; 17, 63797-83-1; 18, 57668-96-9; 19, 63797-84-2; 20, 63797-85-3; 21, 63797-86-4; 22, 63797-87-5; 23, 63797-88-6; 24, 27944-71-4; 25, 63797-89-7; 26, 63797-90-0; 27, 63797-91-1; carboethoxyethylidenetriphenylphosphorane, 1099-45-2; diethyl 5-methyl-3,6-dihydropyran-2,2-biscarboxylate, 63797-92-2.

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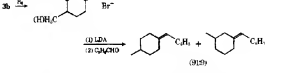

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Exhibit G

Anal. Calcd for $C_{18}H_{14}O_3$: C, 75.76; H, 7.42; mol wt, 190. Found: C, 75.56; H, 7.49; mol wt, 190 (mass spectroscopy), 189 (titration).

Photolysis of 4,6-Dimethylbenzocyclobutenone *p*-Tosylhydrazone Sodium Salt.—The salt prepared from 3.14 g (0.01 mol) of 4,6-dimethylbenzocyclobutenone *p*-tosylhydrazone and 0.5 g (0.011 mol) of 54% sodium hydride dispersion in mineral oil was suspended in 250 ml of dry dioxane and irradiated for 30 min at 25° using a Hanovia 550-W mercury vapor insertion lamp. The reaction mixture was poured into 400 ml of water and extracted with three 75-ml portions of pentane and then three 75-ml portions of benzene. The combined organic extracts were dried over calcium chloride. Removal of solvent left 1.6 g of a brown gummy oil which was chromatographed on 80 g of acid-washed alumina. Elution with 8% ether–pentane gave a total of 108 mg of a white solid which melted at 188–189° after two recrystallizations from heptane. The infrared spectrum of this compound exhibited a weak peak at 1705, medium peaks at 1600, 1470, 1320, 1265, and 1040, and two strong peaks at 850 (very strong) and 725 cm^{-1}. The nmr spectrum showed three singlets at τ 3.23, 6.27, and 7.68, with a ratio of 0.98:1.00:3.09, respectively. Mass spectral analysis gave a molecular weight of 260. The material was assigned the structure of 1,1′-bi(4,6-dimethylbenzocyclobutylidene) on the basis of its elemental analysis and spectra.

Anal. Calcd for $C_{20}H_{20}$: C, 92.26; H, 7.74; mol wt, 260. Found: C, 91.96; H, 8.08; mol wt, 260 (mass spectroscopy).

Elution with 25% ether gave 0.2 g of a yellow oil which could not be induced to crystallize. The infrared spectrum showed peaks at 3020, 2900, 1610, 1500, 1130, and 890 cm^{-1}, indicating that both aromatic and ether groups were present. This material was not characterized further. With 50–75% ether, a total of 0.4 g (14%) of 4,6-dimethylbenzocyclobutenyl *p*-tolyl sulfone, mp 102–104°, was isolated. The infrared spectrum of this material was identical with that of the product obtained by pyrolysis; a mixture melting point of the two samples was not depressed.

Registry No.—4,6-Dimethylbenzocyclobutenone, 6670-28-6; 4,6-dimethylbenzocyclobutenone (azine derivative), 20643-22-5; **1a**, 20643-23-6; **2**, 20678-94-8; **3**, 20643-24-7; **4**, 20643-25-8; **6**, 20643-26-9.

The Sulfation of Hydroxamic Acids

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The reaction of hydroxamic acids with sulfur trioxide–tertiary amine complexes proceeds by O sulfation to give crystalline water-soluble *t*-ammonium N-acylhydroxylamine-O-sulfonates. The reaction of a solution of any of these salts with a base generates an isocyanate function *in situ*. If the base employed is a primary or secondary amine, urea derivatives are obtained in good yield. Carbamates are formed by the decomposition of the salts with phenoxide ion. Aliphatic isocyanates are prepared in good yield by treatment of the salts with tertiary amine in the presence of an inert solvent.

The Lossen rearrangement is a useful method for the preparation of organic isocyanates.[1] This conversion is accomplished by treating a hydroxamic acid with a dehydrating agent such as acetic anhydride, phosphorus pentoxide, or a carbodiimide,[2] forming an isocyanate. The reaction proceeds through an O-ester intermediate which is converted either thermally or by base treatment to an isocyanate and the acid corresponding to the O ester or its conjugate base. The ease of the rearrangement has been shown to be directly related to the acidity of the departing acid.[3]

The preparation of sulfate esters of hydroxamic acids has been examined. These products have been found to undergo a facile Lossen rearrangement to form isocyanates.

Discussion

Sulfation Reactions.—Treatment of benzohydroxamic acid with trimethylamine–sulfur trioxide gave a crystalline, water-soluble product in moderate yield.

$$C_6H_5CONHOH + R_3N{:}SO_3 \longrightarrow C_6H_5CONHOSO_3HNR_3$$
$$\text{I, } R_3 = (CH_3)_3$$
$$\text{II, } R_3 = C_5H_5$$

Elemental analysis and the nmr spectrum of this material indicated a 1:1 adduct. The latter in deuterium oxide contained a nine-proton singlet at 2.68 ppm [DN$(CH_3)_3^+$] and a five-proton multiplet (C_5H_5) centered at 7.45 ppm. The infrared spectrum contained bands at 3225 cm^{-1} (NH), 1663 cm^{-1} (C=O), and 1200 and 1256 cm^{-1} (O=S=O).[4] Dissolution of the adduct in 5% ammonium hydroxide solution at room temperature gave an 82% yield of phenylurea, while treatment with hot 1.5 *N* potassium hydroxide solution gave a 68% yield of *sym*-diphenylurea. These data are consistent with the O-sulfation product (I).

A similar reaction was performed with the more reactive sulfating agent pyridine–sulfur trioxide.[5] The adduct (II) was obtained in 80% yield, employing conditions which were far milder than those required with the trimethylamine reagent. Owing to the high reactivity of this complex, further studies of the scope of the sulfation reaction were conducted with this material.

A number of other mono- and dihydroxamic acids have been treated with pyridine–sulfur trioxide to form the pyridinium N-acylhydroxylamine-O-sulfonates in yields of 70 to 89%. The data are summarized in Table I. With the exception of the stearic acid derivative, all of the salts are water soluble and quite stable in aqueous solution.[6] Another characteristic of these salts is the presence in their infrared spectra of two strong bands between 1200 and 1300 cm^{-1}. Generally, they occur at 1250 and 1300 ± 10 cm^{-1}. These are attributed to the symmetric and asymmetric stretching vibrations of the sulfonic acid function.[4]

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(6) A 10% aqueous solution of II underwent less than 5% decomposition during 10 days at room temperature.

TABLE I

SULFATION OF HYDROXAMIC ACIDS

$$RCONHOH + C_5H_5N{:}SO_3 \longrightarrow RCONHOSO_3HNC_5H_5$$

R	Yield, %	Mp, °C	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
C_6H_5	75	140–141	48.64	4.08	9.45	48.23	4.32	9.26
p-Cl-C_6H_4[a]	88	172–174	43.57	3.35	8.47	43.81	3.47	8.40
$CH_2{=}C(CH_3)$[b]	70	100–110	41.83	4.64	10.76	41.28	4.60	10.33
n-C_7H_{15}[c]	76	109–110	49.03	6.96	8.79	48.96	6.82	8.46
n-$C_{11}H_{23}$[c]	85	119–121	54.55	8.02	7.50	54.44	8.08	7.44
n-$C_{17}H_{35}$[c]	71	116–117	60.22	9.23	6.10	60.16	9.32	6.01
$CH_2{=}CH(CH_2)_8$[d]	75	111–112	53.60	7.25	7.82	53.75	7.49	7.79
$(CH_2)_4$[e]	75	156–157	38.86	4.44	11.33	38.77	4.39	11.24
$(CH_2)_5$[e]	95	160–161	41.37	5.01	10.72	40.99	5.17	10.84

[a] Reference 8. [b] H. Smith, British Patent 852,176 (Oct 26, 1960). [c] Y. Inone and H. Yukawa, *Bull. Chem. Soc. Jap.*, 16, 510 (1940); *Chem. Abstr.*, **35**, 731[8] (1941). [d] G. Kurono, T. Sakai, and Y. Kagotam, *Yakugaku Zasshi*, **84**, 463 (1964); *Chem. Abstr.*, **61**, 4968 (1964). [e] C. D. Hurd and D. G. Batteron, *J. Org. Chem.*, 11, 207 (1946).

The preference for O sulfation of hydroxamic acids can be anticipated from a consideration of the reactivity of these substrates with acylating and sulfonating agents.[7]

Preparation of Ureas and Carbamates.—The behavior of I with potassium and ammonium hydroxide indicated that phenyl isocyanate was being formed in solution. Upon generation of the anion (III), isocyanate should form rapidly by loss of sulfate.[8]

$$\underset{\text{III}}{C_6H_5CONOSO_3^-} \longrightarrow C_6H_5NCO + SO_4^{2-}$$

When a salt was added to an aqueous solution containing 2 to 3 equiv of primary or secondary amine, the isocyanate formed was intercepted by the amine, yielding a urea derivative. Representative amines used in

$$RCONHOSO_3HNR'_3 + R''R'''NH \longrightarrow RNHCONR''R'''$$

this reaction include methyl, *n*-butyl, dimethyl, and diethyl. With multifunctional amines such as ethanolamine and D-glucamine, urea derivatives were formed to the virtual exclusion of urethans. With ethylenediamine, a diurea was formed. In some instances, such as ethylenediamine and D-glucamine, it was not desirable to use an excess of amine. In these cases, a tertiary amine such as triethyl- or trimethylamine was added to assist isocyanate formation. A total of 20 examples of urea formation using this procedure have been recorded. The yields range from 60 to 93% with the majority being 80% or greater (see Experimental Section).

The addition of these salts to an aqueous solution of phenoxide ion gave the corresponding phenyl carbamates.

$$RCONHOSO_3HNR_3' + C_6H_5OK \longrightarrow RNHCOOC_6H_5$$

The behavior of the methacrylic acid derivative (IV) in basic media was examined. When this material was added to potassium hydroxide solution, an 82% yield of acetone, isolated as its 2,4-dinitrophenylhydrazone derivative, was obtained. The rearrangement generated a vinyl isocyanate derivative which then underwent hydrolysis to form acetone.

(7) F. M. Hershenson, L. Bauer, and K. F. King, *J. Org. Chem.*, **33**, 2543 (1968), and references cited therein.

(8) B. E. Hackley, Jr., R. Plapinger, M. Stolberg, and T. Wagner-Jauregg, *J. Amer. Chem. Soc.*, **77**, 3651 (1955).

The decomposition of IV with diethylamine gave a 61% yield of urea derivative (V). The nmr spectrum

$$CH_2{=}C(CH_3)CONHOSO_3HC_5H_5N + R_2NH \longrightarrow$$

IV

$(CH_3)_2C{=}NCONR_2$

V, R = C_2H_5

VI, R = $CH_2CH{=}CH_2$

$$(CH_3)_2C{=}NCONR_2 \rightleftharpoons CH_2{=}C(CH_3)NHCONR_2$$

of this material indicated that it existed exclusively in the form of the C=N tautomer. The spectrum (neat) contained a pair of overlapping triplets at 1.03 and 1.13 ppm (J = 7.0 Hz) and a pair of overlapping quartets at 3.19 and 3.38 ppm (J = 7.0 Hz). These are assigned to the methyl and methylene groups, respectively, of the N-ethyl groups. Their magnetic nonequivalence is due to restricted rotation about the nitrogen–carbonyl bond.[9] A pair of singlets at 1.97 and 2.03 ppm are also present in the spectrum. These are assigned to the methyl groups of the isopropylidene function. This nonequivalence results from *syn* and *anti* isomerism about the imine double bond.

The position of the equilibrium between the tautomers was found to be solvent independent when examined by nmr. The spectra were recorded in carbon tetrachloride, deuterated acetone, and deuterated dimethyl sulfoxide. No change was noted in the spectra except that the methyl groups of the isopropylidene coalesced to a singlet at 2.00 ppm. Contributions from the vinylurea tautometer were not found in any of the spectra.

The urea derivative (VI) from diallylamine was formed in 63% yield. The equilibrium position of this product was the same as that of the diethyl derivative on the basis of nmr data.

The thermal isomerization of vinylureas to their ketimine derivatives has been demonstrated by Sato.[10] Since the infrared spectra of crude products before distillation are essentially identical with that of the pure product, the isomerization is probably also base catalyzed owing to the presence of pyridine and excess amine in the reaction mixture. Attempts to prepare a similar derivative using ethylenimine were unsuccessful. The crude product was quite unstable, and it decomposed to a dark brown resinous solid.

Preparation of Isocyanates.—The *in situ* formation of an isocyanate by treatment of a sulfated hydroxamic

(9) R. H. Bible, "Interpretation of NMR Spectra: An Empirical Approach," Plenum Press, New York, N. Y., 1965, p 66.

(10) M. Sato, *J. Org. Chem.*, **26**, 770 (1961).

acid with a tertiary amine was employed in the preparation of certain carbamate and urea derivatives. A similar technique was employed for the isolation of the isocyanates. The sulfated hydroxamic acid was treated with a tertiary amine in aqueous solution in the presence of an inert solvent such as petroleum ether or carbon tetrachloride. As the isocyanate was formed it was extracted into the organic layer. Work-up of the reaction gave the crude isocyanate, which was purified by distillation.

$$RCONHOSO_3H.C_5H_5N + R'_3N \longrightarrow RNCO + C_5H_5N(NR'_3)HSO_4$$

This procedure is quite satisfactory for the preparation of aliphatic isocyanates. Yields ranging from 63 to 75% were obtained. The fatty acid derivatives often produced emulsions, which made it difficult to separate the organic phase. In most cases, this problem was overcome by addition of a small amount of acetonitrile to the reaction mixture. With the stearic acid derivative, however, the problem was too severe, and prevented isolation of heptadecyl isocyanate. The infrared spectrum of the crude mixture showed bands assignable to isocyanate and urea.

The preparation of phenyl isocyanate by this procedure gave only a 20% yield of product. In addition, a 45% yield of *sym*-diphenylurea was also formed. These data reflect the greater reactivity of aromatic *vs.* aliphatic isocyanates.

A number of unsuccessful attempts were made to prepare aliphatic diisocyanates by this method. In all cases, polymeric materials were obtained.

Experimental Section

Nuclear magnetic resonance spectra were run on a Varian A-60 spectrometer in the appropriate solvent, with tetramethylsilane as an internal or external standard. Infrared spectra were recorded on a Beckman Model IR-12 or a Perkin-Elmer 137 infrared spectrophotometer. Melting points were obtained using a Thomas–Hoover melting point apparatus and are uncorrected.

Preparation of Starting Materials.—The hydroxamic acids were prepared according to literature procedures. The pyridine sulfur trioxide was prepared according to the method of Baumgarten.[11] The trimethylamine sulfur trioxide was obtained from the American Cyanamid Co., New York 20, N. Y.

Sulfation of Benzohydroxamic Acid with Trimethylamine–Sulfur Trioxide.—A mixture of 40.0 g (0.29 mol) of benzohydroxamic acid and 40.0 g (0.29 mol) of trimethylamine–sulfur trioxide was slurried in 300 ml of acetone at ambient temperature for 5 days. The mixture was filtered and evaporated to dryness. The residue was dissolved in 200 ml of hot ethanol and cooled to give 33 g (0.12 mol, 41%) of trimethylammonium N-benzoylhydroxylamine-O-sulfonate (I), mp 150–154°. An analytical sample was prepared by recrystallization from ethanol: mp 155–156°.

Anal. Calcd for $C_{10}H_{16}N_2O_5S$: C, 43.46; H, 5.83; N, 10.13. Found: C, 43.38; H, 5.95; N, 10.12.

General Procedure for the Sulfation of Hydroxamic Acids with Pyridine–Sulfur Trioxide.—A solution of 0.1 mol of hydroxamic acid (or 0.22 mol for dihydroxamic acids) and 0.11 mol of pyridine–sulfur trioxide in 150 ml of acetonitrile was refluxed with stirring for 0.5 to 4.0 hr. The solvent was evaporated at aspirator pressure and the residue was purified by recrystallization from ethanol or acetonitrile. Similar yields may be obtained by keeping the reaction mixture at room temperature for about 18 to 24 hr. With the 10-undecylenic acid derivative, this latter procedure was preferred.

Preparation of Ureas.—The sulfated hydroxamic acid was added portionwise or as a 30–50% aqueous solution to a stirred solution of 2 to 3 equiv of ammonia, or primary or secondary amine in water. (The amine concentration in the aqueous solution was about 5–10% by weight.) The reaction temperature was maintained at 20–30° by external cooling. After addition was complete, the mixture was stirred at ambient temperature for 0.5 hr and filtered. The residue was washed with water and air dried. Using this procedure, the following ureas were prepared: $C_6H_5NHCONH_2$, mp 146–148°, 82%; $C_6H_5NHCON(C_2H_5)_2$, mp 84–85°, 75% (trimethylammonium salt), 89% (pyridinium salt); $C_6H_5NHCONHCH_2$-*n*, mp 126–127°, 83% (trimethylammonium salt), 94% (pyridinium salt); $C_6H_5NHCONHCH_2I_2$, mp 127–128°, 87% (trimethylammonium salt); $C_6H_5NHCONHCH_2CH_2OH$, mp 122–123°, 72% (pyridinium salt); *n*-C_7H_{15}-$NHCONHCH_2$-*n*, mp 56–57°, 78%; *p*-Cl-$C_6H_4NHCONH$-CH_2-*n*, mp 172–174°, 86%; *p*-Cl-$C_6H_4NHCON(C_2H_5)_2$, mp 114–116°, 88%; *n*-$C_{12}H_{25}NHCONHCH_3$, mp 66–67°, 81%; *n*-$C_{17}H_{35}NHCONHCH_3$, mp 99–100°, 89%; $CH_2{=}CH{-}(CH_2)_8$-$NHCONHCH_2$, mp 69–70°, 82%; $[(CH_3)_2NHCONHCH_2]_2$, mp >280°, 79%; $[(CH_3)_2NHCONHCH_2]_3$, mp 201–203°, 91%; $[(CH_3)_2NHCONHCH_2$-*n*$]_3$, mp 192–194°, 83%.

Preparation of 1,1-Diethyl-3-phenylurea Using Trimethylamine as Catalyst.—Pyridinium N-benzoylhydroxylamine-O-sulfonate (3.0 g) was added portionwise to a stirred solution of 4.8 g of 25% trimethylamine in methanol and 1.0 g of diethylamine in 18 ml of water maintained at 20–25° by a cooling bath. After addition was complete, the mixture was stirred at room temperature for 0.5 hr and filtered. The residue was washed with water and air dried to give 1.4 g of product (75%), mp 84–86°. Using the same type of procedure $[C_6H_5NHCONHCH_2]_3$, mp 276–277°, and *p*-gluco-$C_6H_5NHCONHCH_2(CHOH)_4CH_2OH$, mp 173–174°, were obtained in yields of 61 and 71%, respectively.

Preparation of Phenyl Carbanilate.—Trimethylammonium N-benzoylhydroxylamine-O-sulfonate (3.0 g) was added portionwise at room temperature to a stirred solution of 5.8 g of potassium phenoxide in 25 ml of water. After addition was complete, the mixture was stirred at room temperature for 0.5 hr, filtered, and air dried to give 1.85 g (79%) of product mp 122–123°. Using this procedure, *n*-$C_7H_{15}NHCOOC_6H_5$, mp 36–37°, was prepared in 71% yield.

Reaction of Pyridinium N-Methacryloylhydroxylamine-O-sulfonate with Potassium Hydroxide.—Compound IV, 2.6 g (0.01 mol), was added portionwise with cooling and stirring to 10 ml of 3 *N* potassium hydroxide solution. After addition was complete, this solution was added slowly with cooling and stirring to a solution of 3.0 g of 2,4-dinitrophenylhydrazine in 15 ml of concentrated sulfuric acid, 25 ml of water, and 50 ml of absolute ethanol. The solution was then chilled and filtered to give 1.95 g (82%) of acetone 2,4-dinitrophenylhydrazone, mp 122–124°. A mixture melting point with an authentic sample was undepressed.

Preparation of 1,1-Dialkyl-3-isopropylideneureas.—A 30% aqueous solution of IV was added dropwise to a vigorously stirred solution of 3 equiv of secondary amine in 200 ml of chloroform cooled in an ice water bath. After addition was complete, the chloroform layer was separated and the aqueous layer was extracted with chloroform. The chloroform extracts were combined, dried, and evaporated to give the crude product, which was purified by vacuum distillation. The following ureas were prepared.

(a) 1,1-Diethyl-3-isopropylideneurea[12] (61%): bp 122° (19 mm); $n^{25}D$ 1.4562.

Anal. Calcd for $C_8H_{16}N_2O$: N, 17.93. Found: N, 17.87.

(b) 1,1-Diallyl-3-isopropylideneurea (63%): bp 65° (0.05 mm); $n^{25}D$ 1.4790; nmr (neat) 2.00 [s, 6 H, $(CH_3)_2C{=}$], 3.83 and 4.05 (d, 4 H, J = 5.0 Hz, CH_2N), and 4.90–6.30 ppm (complex m, 6 H).

Anal. Calcd for $C_{10}H_{16}N_2O$: C, 66.62; H, 8.95; N, 15.54. Found: C, 66.25; H, 8.87; N, 15.88.

General Procedure for the Preparation of Isocyanates.—A 25% aqueous solution containing 0.25 mol of trimethylamine was added dropwise to a vigorously stirred mixture of a solution of 0.1 mol of N-acylhydroxylamine-O-sulfonic acid or -ammonium salt in 150 ml of water and 250 ml of carbon tetrachloride or petroleum ether cooled in an ice bath. After addition was complete, the mixture was stirred at ice-bath temperature for 0.25 hr and then diluted with water. A 100- to 150-ml portion of acetonitrile was added (this was not necessary in the preparation of *n*-heptyl isocyanate or phenyl isocyanate) and the organic layer was separated. The aqueous phase was reextracted with the organic

(11) P. Baumgarten, *Ber.*, **59**, 1166 (1926).

(12) J. B. Dickey, U. S. Patent 2,592,254 (April 8, 1952).

solvent, and the extracts were combined, dried over anhydrous sodium sulfate, filtered, and evaporated to yield the crude isocyanate, which was purified by distillation. The following isocyanates were prepared by this procedure: (a) *n*-heptyl[12] (63%), bp 83–84° (23 mm), n^{25}D 1.4326; (b) *n*-undecyl[13] (54% from petroleum ether, 75% from carbon tetrachloride), bp 144° (18 mm), n^{25}D 1.4358; (c) 9-decenyl[13] (70%), bp 127–128° (17 mm), n^{25}D 1.4446; (d) phenyl (20%), identified by gpc and infrared spectroscopy.

Registry No.—I, 20633-41-4; $C_4H_9NHCONH_2$, 64-10-8; $C_8H_{17}NHCON(C_2H_5)_2$, 1014-72-8; $C_8H_{17}NHCONH$-C_8H_{17}-*n*, 3083-88-3; $C_2H_5NHCON(CH_3)_2$, 101-42-8; $C_6H_5NHCONHCH_2CH_2OH$, 3747-47-5; *n*-$C_7H_{15}NHCONHC_7H_{15}$-*n*, 20633-46-9; *p*-$ClC_6H_4NHCONHC_6H_{13}$-*n*, 6333-41-1; *p*-$ClC_6H_4NHCON(C_2H_5)_2$, 15737-37-8; *n*-$C_{11}H_{23}NHCONHCH_3$, 20633-56-1; *n*-$C_{17}H_{35}$-$NHCONHCH_3$, 20633-49-2; $CH_2{=}CH{=}(CH_2)_8NHCONHCH_3$, 20633-50-5; $[(CH_2)_2NHCONHCH_3]_2$, 20633-51-6; $[(CH_2)_2NHCONHCH_3]_3$, 20633-52-7; $[(CH_2)_2NHCONHC_7H_{15}$-$n]_3$, 20633-53-8; II, 20633-55-0; $[C_5H_9NHCONHCH_2]_2$, 849-97-8; D-gluco-C_6H_9-$NHCONHCH_2(CHOH)_4CH_2OH$, 20642-67-5; phenyl carbanilate, 4930-03-4; *n*-$C_7H_{15}NHCOOC_2H_5$, 2594-41-4; 1,1-diallyl-3-isopropylideneurea, 20642-56-2.

(13) V. E. Shashoua, W. Sweeney, and R. F. Tietz, *J. Amer. Chem. Soc.*, **82**, 866 (1960).

The Effect of the Imidazole Group on the Hydrolysis of N-[2-(4-Imidazolyl)ethyl]phthalimide[1a]

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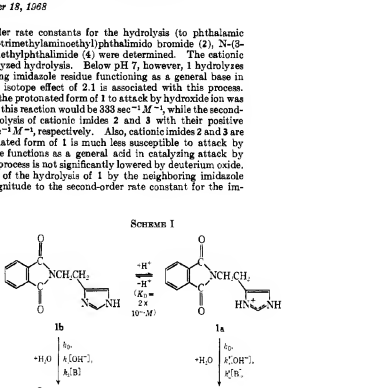
The pH dependencies (at 25°) of the pseudo-first-order rate constants for the hydrolysis (to phthalamic acids) of N-[2-(4-imidazolyl)ethyl]phthalimide (1), N-(2-trimethylaminoethyl)phthalimido bromide (2), N-(3-trimethylaminopropyl)phthalimido bromide (3), and N-methylphthalimide (4) were determined. The cationic imides 2 and 3 are most susceptible to hydroxide ion catalyzed hydrolysis. Below pH 7, however, 1 hydrolyzes most rapidly. This effect was ascribed to the neighboring imidazole residue functioning as a general base in catalyzing attack by water. A deuterium oxide solvent isotope effect of 2.1 is associated with this process. The possibility that this effect reflects the susceptibility of the protonated form of 1 to attack by hydroxide ion was deemed unlikely, since the second-order rate constant for this reaction would be 333 sec^{-1} M^{-1}, while the second-order rate constants for the hydroxide ion catalyzed hydrolysis of cationic imides 2 and 3 with their positive charges closer to the carbonyl carbon atom are 91 and 41 sec^{-1} M^{-1}, respectively. Also, cationic imides 2 and 3 are susceptible to direct attack by water, whereas the protonated form of 1 is much less susceptible to attack by water. It is unlikely that a neighboring imidazole residue functions as a general acid in catalyzing attack by hydroxide ion, since the calculated rate constant for this process is not significantly lowered by deuterium oxide. The first-order rate constants for intramolecular catalysis of the hydrolysis of 1 by the neighboring imidazole group (2.9×10^{-4} sec^{-1}) was found to be similar in magnitude to the second-order rate constant for the imidazole-catalyzed hydrolysis of 4 (2.0×10^{-4} sec^{-1} M^{-1}).

Neighboring amide groups are potent nucleophiles, and under physiological conditions amide groups can enhance the rate of hydrolysis of adjacent ester and amide residues by several orders of magnitude.[2] Often, the rate-limiting step in these reactions is the hydrolysis of the imide intermediate. Because of the possible importance of the amide group in enzymically catalyzed hydrolytic reactions, we have investigated the effect of the imidazole group on the hydrolysis of N-[2-(4-imidazolyl)ethyl]phthalimide (1) (Scheme I).

Scheme I



Experimental Section

Materials.—N-Methylphthalimide was obtained from Eastman Organic Chemicals and recrystallized twice from 95% ethanol: mp 134–135° cor (lit.[4] mp 133–134°).

N-(2-Trimethylaminoethyl)phthalimido bromide was prepared by mixing 5.08 g (20 mmol) of N-(2-bromoethyl)phthalimide (from Eastman Organic Chemicals) dissolved in 75 ml of dioxane with 5.4 g (91 mmol) of trimethylamine (from Eastman Organic Chemicals) dissolved in 150 ml of dioxane. After the mixture stood overnight at room temperature, the precipitate was collected and recrystallized three times from 95% ethanol: dec pt 290–291° cor. *Anal.* Calcd for $C_{13}H_{17}N_2O_2Br$: C, 49.85; H, 5.47; N, 8.95; Br, 25.52. Found: C, 49.59; H, 5.46; N, 8.75; Br, 25.20.

N-(3-Bromopropyl)phthalimide was prepared from potassium phthalimide (from Eastman Organic Chemicals) and 1,3-dibromopropane (from CalBiochem) according to the method of Gabriel:[4] mp 71–73° cor (lit.[4] mp 72–73°).

N-(3-Trimethylaminopropyl)phthalimido bromide was prepared by mixing 2.68 g (10 mmol) of N-(3-bromopropyl)phthal-

(1) (a) This study was supported by a grant (AM-09276) from the National Institutes of Health, U. S. Public Health Service. (b) To whom inquiries regarding this work should be made.

(2) S. C. K. Su and J. A. Shafer, *J. Org. Chem.*, in press, and references therein.

(3) M. Freund and H. Beck, *Ber.*, **27**, 1042 (1904).

(4) S. Gabriel and J. Weiner, *ibid.*, **21**, 2669 (1888).

Exhibit H

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, PHOSPHATE DIVISION, MONSANTO CHEMICAL COMPANY]

Phosgene Derivatives. The Preparation of Isocyanates, Carbamyl Chlorides and Cyanuric Acid[1]

BY R. J. SLOCOMBE, EDGAR E. HARDY, J. H. SAUNDERS AND R. L. JENKINS

At the time the major portion of this work had been completed the vapor phase reaction between phosgene and amines had not been described.[2] However, a somewhat similar reaction was reported recently.[3] The use of catalysts, preheating of the phosgene, and relatively long sojourn times (2.3–17.7 sec.) were recommended. Work reported here more closely defines the conditions of the reaction and shows that the recommendations cited above are not necessary.

This process has been made the basis of a general synthetic procedure for the preparation of carbamyl chlorides and isocyanates.

The development of this procedure was prompted by a study of the preparation of methylcarbamyl chloride by passing phosgene over molten methylamine hydrochloride.[4,5] Experiments showed that the yield of isocyanate was adversely affected when the area of contact was increased by dispersing phosgene beneath the surface of the molten salt. This observation suggested that the reaction involved not only the liquid–gas interfacial contact, but also depended upon conditions in the gas phase. It was reasoned that some of the methylamine hydrochloride was vaporized and dissociated and that the free amine may then have reacted with phosgene. If this were true, a direct reaction between methylamine and phosgene in the vapor-phase should be feasible. Such a reaction was carried out successfully, and methylcarbamyl chloride was obtained, which was converted by more orthodox methods to the isocyanate in 88% over-all yield.

The vapor-phase method for the preparation of methyl isocyanate was found to have wide applicability. A variety of mono- and disubstituted carbamyl chlorides and isocyanates were prepared in 70 to 90% yield by the reaction between phosgene and primary or secondary amines at 275°.

$$RNH_2 + COCl_2 \longrightarrow RNHCOCl + HCl$$
$$RNHCOCl \rightleftharpoons RNCO + HCl$$
$$R_2NH + COCl_2 \longrightarrow R_2NCOCl + HCl$$

The isocyanate was obtained from the monosubstituted carbamyl chloride by treatment with a tertiary amine. Those isocyanates which boil above 120° were also liberated by refluxing the corresponding monosubstituted carbamyl chloride in benzene or toluene.

The method is applicable apparently to the use of any primary or secondary monoamine which can be boiled readily without decomposition. For practical purposes the procedure is limited to amines containing from one to twelve carbon atoms. Only two conditions were found necessary for the satisfactory operation of the vapor-phase reaction. These were, (1) the amine vapor must be adequately mixed with phosgene, and (2) the reaction zone must be hot enough to avoid the separation of reaction products from the gas phase in that zone.

Similarly, at 500° ammonia was converted to carbamyl chloride in almost quantitative yield.

$$NH_3 + COCl_2 \longrightarrow H_2NCOCl + HCl$$

The reaction between ammonia and phosgene at 400° was mentioned by Rupe and Labhard,[6] but no experimental details were given. When this reaction was run at 400°, considerable amounts of ammonium chloride were formed. In contrast, at 500° this undesirable by-product was almost entirely eliminated. The carbamyl chloride was readily converted to cyanuric acid by heating to 180°

$$3H_2NCOCl \longrightarrow (HNCO)_3 + 3HCl$$

A consideration of the temperatures used in the vapor-phase reaction and in the thermal decomposition of monosubstituted carbamyl chlorides[4,5] indicates that the isocyanate is the end-product of the vapor-phase reaction at 275° when primary amines are employed, but, upon cooling, the isocyanate may combine with hydrogen chloride to give the carbamyl chloride.

That this sequence is true was demonstrated by removing the hydrogen chloride from the products of the reaction before combination with the isocyanate could occur. As typical examples, methyl, phenyl and octyl isocyanates were prepared directly without the intermediate formation of the carbamyl chlorides.

Experimental

General Method for the Preparation of Carbamyl Chlorides.—The apparatus is shown in Fig. 1. The reactor (K) was made of 15-mm. I. d. Pyrex tubing. The heated portion was 15 in. long and wound with $^1/_{18}$-in. nichrome ribbon having a resistance of 15 ohms. Phosgene entered the reaction zone (M) through a 6-mm. tube (A) which was drawn out to a 1-mm. tip. Amine vapor entered the reaction zone through the annular space between the phosgene inlet tube and the reactor tube. Indentations were made in the walls of the reactor to impart added turbulence to the gases.

(1) The work described in this paper was done in part under Contract OEM-sr-845, recommended by the National Defense Research Committee, between the Office of Scientific Research and Development, and the Monsanto Chemical Company. A portion of this work has been described by Jenkins and Hardy in Off. Pub. Bd. Report, P. B. 27396, "The Preparation of Isocyanates, Cyanuric Acid, and Decontaminant 40."

(2) Saunders and Slocombe, *Chem. Revs.*, **43**, 203 (1948).

(3) Modersohn, "Action of Phosgene on Primary and Secondary Amines in the Gas Phase," Off. Pub. Bd., Report PB 707.

(4) Gattermann and Schmidt, *Ber.*, **20**, 118 (1887).

(5) Gattermann, *Ann.*, **244**, 29 (1888).

(6) Rupe and Labhard, *Ber.*, **33**, 233 (1900).

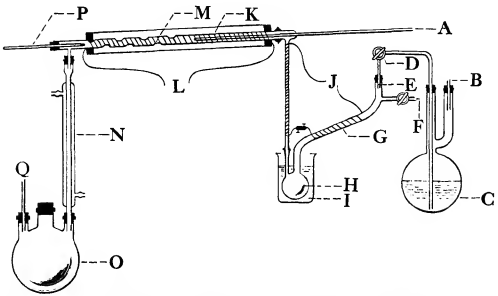


Fig. 1.—Vapor-phase reaction apparatus: A, phosgene inlet from flowmeter; B, nitrogen inlet to amine feed flask; C, amine feed flask; D, amine flow control cock; E, calibrated tip for amine addition; F, nitrogen inlet for sweeping; G, preheater section of vaporizer; H, flash vaporizer; I, salt-bath heated with bunsen burner; J, electrical leads to vaporizer heaters; K, reactor tube; L, electrical leads to reactor heater; M, reactor zone; N, product condenser; O, receiving flask; P, thermometer for reactor outlet temperature; Q, off-gas outlet.

The salt-bath (I) for the flash vaporizer (H) was heated to a temperature at least 100° above the boiling point of the amine being used. A weighed amount of the desired amine, if a liquid at room temperature, was charged into the amine feed flask (C). Nitrogen pressure ($^1/_2$ to $^1/_4$ p. s. i. from a cylinder reducing valve) was applied to the amine feed flask at (B) to force the liquid to the stopcock (D) which controlled the rate of amine flow. If the amine was a gas at room temperature it was fed from a cylinder through an orifice-type flow meter. In such cases the amine vaporizer was not used.

The reactor (K) was heated to a temperature of 275°, read at (P), while a slow stream of nitrogen was sweeping through the system from the nitrogen inlet (F) on the side of the amine preheater (G). The phosgene was turned on to the desired rate as shown by an orifice-type flowmeter. The amine addition was then begun. The maximum rate of addition of liquid amine was estimated from the rate of drop formation at (E). A phosgene:amine molar ratio of 1.3 was used. The flow of nitrogen was used only when the operation was started, interrupted or terminated. The nitrogen served to prevent phosgene or hydrogen chloride from backing into the amine vaporizing system and forming a deposit of the urea and the amine hydrochloride. Excess phosgene was decomposed with steam.

The limits of the conditions for the reaction are broad. Reaction temperatures of 240–350°, sojourn times of 0.24–2.6 sec., and phosgene–amine molar ratios as low as 1.2 have been used with good success.

Conversion of Carbamyl Chlorides to Isocyanates

(a) **Use of a Tertiary Amine.**—Into a 1-l., 3-necked flask was placed 150 g. of crude methylcarbamyl chloride obtained from 49 g. (1.57 moles) of methylamine. The flask was fitted with a reflux condenser, mechanical stirrer and dropping funnel. A solution of 165 ml. (2.0 moles) of dry pyridine and 165 ml. of dry toluene was added slowly with stirring. Vigorous reaction accompanied this addition. The resulting solution was distilled through an 18-in., helix-packed column. The yield of methyl isocyanate, b. p. 37–39°, was 79 g., 88% based on amine.

Other isocyanates below amyl were obtained by adding dimethylaniline directly to the carbamyl chloride and distilling. One-fourth molar excess of amine was used. Yields and physical properties are given in Table I.

In spite of extreme care to avoid the presence of moisture, crystals frequently occurred in *n*-butyl and *s*-butyl isocyanates when these isocyanates were distilled at atmospheric pressure. Distillation at 20 mm. gave crystal-free products.

n-Butyl isocyanate showed a marked tendency to polymerize in the presence of the tertiary amine hydrochloride. An equal volume of "orthene" (technical *o*-dichlorobenzene) and of dimethylaniline were used to decompose the butylcarbamyl chloride. The final reaction mixture was cooled to freeze out the amine hydrochloride. The supernatant liquid was decanted and distilled with good yields.

(b) **Refluxing with an Inert Solvent.**—The crude phenylcarbamyl chloride obtained from 50 g. of aniline was dissolved in 75 ml. of toluene or benzene and refluxed until no more hydrogen chloride was evolved. On fractional distillation, 55 g. (86%) of phenyl isocyanate was collected, b. p. 158–162°. Other carbamyl chlorides above butyl were converted to the isocyanate by this method. Yields and physical data are given in Table I.

Disubstituted Carbamyl Chloride.—The general procedure was the same as for the monosubstituted carbamyl chlorides. The reaction product was refluxed with one-half its weight of dry toluene to drive out dissolved phosgene and hydrogen chloride. The solvent was removed and the product distilled. Yields and physical data are given in Table I.

Carbamyl Chloride.—The general procedure was the same as that used in the preparation of substituted carbamyl chlorides, with the following exceptions. In the first experiments carbamyl chloride was collected as a colorless liquid. In later experiments the receiver equipped with a reflux condenser and a mechanical stirrer, and containing 1.5 to 2.0 l. of orthene, was attached directly to the vapor-phase reactor. The reaction temperature was 500°.

The undiluted carbamyl chloride showed a marked tendency to solidify below room temperature, and to lose hydrogen chloride and polymerize on standing. When the carbamyl chloride was to be used at once in the synthesis of cyanuric acid, orthene was put in the receiver and stirred to prevent caking of the solid. No yield of pure carbamyl chloride was determined.

TABLE I

PREPARATION OF ISOCYANATES AND DISUBSTITUTED CARBAMYL CHLORIDES

Amine	Boiling range of product, °C. (uncor.)	Yield, %
Methyl	37–39[a,g]	88
Ethyl	58–61[a]	71
n-Propyl	86–88[b]	76
Isopropyl	70–75[c]	82
Allyl	83–85[d]	75
n-Butyl	113–116[e,f]	70
s-Butyl	99–101[g]	70
Isobutyl	100–103[h]	78
Amyl (mixed)	129–140[i]	70
Cyclohexyl	165–168[j]	88
Phenyl	158–162[k]	86
n-Octyl	200–204[l]	81
n-Dodecyl	130–140 (4 mm.)[f]	58
Dimethyl	166–168[m]	90
Di-*n*-butyl	245–247[n]	81
Diamyl (mixed)	115–125 (3 mm.)[o]	83

[a] Wurtz, *Ann. chim. phys.*, [3] 42, 43 (1854); n^{20}D 1.3801; d^{20}_4 0.9039. [f] [b] Oliveri-Mandalà and Noto, *Gazz. chim. ital.*, **43**, I, 514 (1913). [c] Hofmann, *Ber.*, **15**, 752 (1882); n^{20}D 1.3886; d^{20}_0 0.8669. [f] [d] Hofmann, *Ann.*, **102**, 285 (1857). [e] *Anal.*[f] Calcd.: C, 60.58; H, 9.15. Found: C, 60.42; H, 8.84; n^{20}D 1.4060; d^{20}_0 0.880. [f] U. S. Patent 2,326,501. [g] *Anal.*[f] Calcd.: C, 60.58; H, 9.15. Found: C, 60.80; H, 9.02; n^{20}D 1.3989; d^{21}_4 0.8804. [h] Anschutz, *Ann.*, **359**, 202 (1908). [i] Sharples mixed amylamines were used: Custer, *Ber.*, **12**, 1328 (1879). [j] Skita and Rolfes, *ibid.*, **53**, 1242 (1920). [k] Hofmann, *ibid.*, **3**, 653 (1870). [l] *Anal.*[f] Calcd.: C, 69.63; H, 11.04. Found: C, 70.14; H, 11.25; n^{20}D 1.4314; d^{20}_4 0.8768. [m] Michler and Escherich, *Ber.*, **12**, 1162 (1879). [n] Werner, *J. Chem. Soc.*, 115, 1010 (1919). [o] Sharples mixed diamylamines were used: Atkinson, *J. Chem. Soc.*, 105, 1290 (1914).

Cyanuric Acid.—The crude carbamyl chloride was converted to cyanuric acid[8] by heating at about 180° for three hours. The temperature rose slowly from 55 to 180°, as hydrogen chloride was evolved and polymerization progressed. When no solvent was used the cyanuric acid could not be recovered without breaking the flask. When orthene was used as a solvent the product was readily obtained by filtering the resulting slurry and drying the finely divided solid at 200–210°. The yield was 82–89%, based on ammonia. The cyanuric acid, without further processing, was 98% pure, as indicated by a Kjeldahl analysis and solubility in alkali. The isomeric cyamelid is insoluble in dilute alkali.[9]

Anal.[10] Calcd. for $C_3H_3O_3N_3$: N, 32.56. Found: N, 31.9, 31.9.

Solubility of Hydrogen Chloride in Phenyl Isocyanate.—In order to demonstrate the lowest temperature at which phenyl isocyanate can be condensed from the reaction gases without the formation of phenylcarbamyl chloride, the stability of phenylcarbamyl chloride was observed briefly.

(a) **Toluene Solution.**—A 1-l., 3-necked flask was fitted with a sealed mechanical stirrer, a gas inlet tube, a thermometer, and a condenser, which in turn was connected to a trap cooled in a Dry Ice–methanol-bath. A solution of 100 g. (0.84 mole) of phenyl isocyanate in 500 ml. of dry toluene was placed in the flask. The toluene solution was maintained at 50°. Dry hydrogen chloride was bubbled into the solution until a constant weight was reached. Correction was made for any toluene or isocyanate which was condensed in the cold trap. The gain in weight of the solution was taken as the weight of hydrogen chloride absorbed. The process was repeated at 80, 95 and 105°. The data are recorded in Table II.

TABLE II

ABSORPTION OF HYDROGEN CHLORIDE BY 100 G. OF PHENYL ISOCYANATE

Temp., °C.	No solvent		In 500 ml. toluene	
	HCl, g.	RNHCOCl, %	HCl, g.	RNHCOCl, %
50	.....	.....	32.3 ± 0.9	100
80	.....	.....	11.7 ± 0.5	38.2 ± 2
95	11.2 ± 0.1	36.6 ± 0.5	4.5 ± 0.6	14.7 ± 2
105	6.7 ± 0.1	21.9 ± 0.2	1.7 ± 0.3	5.6 ± 1
115	4.0 ± 0.1	13.2 ± 0.3	.....	.....
125	1.9 ± 0.1	6.1 ± 0.4	.....	.....

It is apparent that condensation of phenyl isocyanate in boiling toluene will not be accompanied by the formation of appreciable amounts of phenylcarbamyl chloride.

(b) **No Solvent.**—The procedure described for the determination in toluene solution was followed, except that no solvent was used. The absorption of hydrogen chloride was observed at 95, 105, 115 and 125°. The weight of hydrogen chloride absorbed was determined by titration of the chloride in the isocyanate–carbamyl chloride solution.[10] The data are recorded in Table II. These results are in general agreement with those of Lengfeld and Stieglitz, who reported that phenylcarbamyl chloride dissociates at 100°.[11]

The Direct Preparation of Phenyl Isocyanate.—The procedure for the reaction between phosgene and aniline was the same as that used for carbamyl chlorides, with the following changes. The gaseous products passed from the reactor into a 20-mm. Pyrex tube which was heated electrically to 130°. This tube extended almost to the bottom of a 500-ml. 3-necked flask which contained 250 ml. of refluxing toluene. To the flask was attached an 8-in. bead-packed column, which was heated externally to maintain a temperature of 110° inside the column. A long condenser was placed on top of the column. From 50 g. (0.54 mole) of aniline there was obtained 52.3–56.8 g. (82–89%) of phenyl isocyanate, b. p. 157–162°.

Analysis of the crude reaction mixture before distillation indicated the presence of only traces of chloride.

The Direct Preparation of *n*-Octyl Isocyanate.—The apparatus and procedure were the same as the preceding description for phenyl isocyanate. From 320 g. (2.48 moles) of *n*-octylamine there was obtained 339 g. (88%) of *n*-octyl isocyanate, b. p. 200–207°.

The Direct Preparation of Methyl Isocyanate.—The reactor and method described for carbamyl chlorides were used, with the following modifications. A 20-mm. Pyrex tube led from the reactor to a 3-l. flask which contained 450 g. of dry pyridine and 250 ml. of dry toluene. The connecting tube extended to within 1 in. of the surface of the liquid in the flask, and was heated electrically to 140°. An 8-in. Vigreux column was attached to one neck of the flask. On top of this column was placed an 8-in. helix-packed column, partial take-off head, and condenser, which was connected to a Dry Ice–methanol trap. The pyridine–toluene mixture was boiled vigorously. During the reaction distillate boiling at 60–80° was collected from the partial take-off head. When the reaction was completed the distillation was continued until the distillate boiled at 112°. Redistillation of this crude product gave methyl isocyanate, b. p. 37–41°. From 36 g. (1.16 moles) of methylamine there was obtained 40 g. of methyl isocyanate, 60.7%, based on amine. Essentially no methylcarbamyl chloride was formed, since the pyridine combined

(7) We are indebted to the Clark Microanalytical Laboratory, Urbana, Illinois, for the analytical determinations.

(8) Rupe and Metz, *Ber.*, **36**, 1092 (1903).

(9) Hantzsch, Hofmann and Lehmann, *ibid.*, **35**, 1013 (1905).

(10) The authors wish to thank Mr. W. B. Dunlap for this analysis.

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with the hydrogen chloride at a temperature above that at which methylcarbamyl chloride dissociates (93–94°).[6]

Summary

1. The vapor-phase reactions of primary and secondary monoamines with an excess of phosgene were investigated. A new method for the preparation of mono- and disubstituted carbamyl chlorides and of monoisocyanates from aliphatic, olefinic, alicyclic and aromatic amines is described.

2. Carbamyl chloride was prepared by the reaction between phosgene and ammonia at 500°. The carbamyl chloride was of high purity and was readily converted to cyanuric acid by heating.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ST. LOUIS UNIVERSITY]

Polarographic, Potentiometric and Conductometric Studies on the Aspartate and Alaninate Complexes of Copper

BY NORMAN C. LI AND EDWARD DOODY[1]

As a part of a general program on complex formation between different metallic and amino acid ions[2] this paper[3] presents the results on the aspartate and alaninate complex of copper. The methods used were polarographic, potentiometric and conductometric.

Several investigators have studied the complex ions formed between cupric and alaninate ions. Boorsook and Thimann[4] have deduced from spectrophotometric and electrometric data that the complex formed in alkaline solutions was CuA_3^-. Riley and co-workers[5,6] using a cupric ion concentration cell, have concluded that a very stable complex ion of the type CuA_3^- is formed; however, the data for alaninate solutions reported in the two papers do not agree. Using the dropping mercury electrode, Keefer[7] has reported the composition and stability of the alaninate complex of copper and has found the complex to be CuA_2, but it is desirable to extend the range of his studies to lower pH values by using buffer solutions.

Experimental

Reagent quality chemicals were used without further purification except for the alanine which was once recrystallized. A standard solution of cupric nitrate was prepared by dissolving about 0.025 mole of cupric nitrate in 250 ml. of water. To this solution excess potassium iodide was added and the liberated iodine was titrated with a standard solution of sodium thiosulfate to the starch endpoint. Stock solutions of the potassium aspartate and potassium alaninate were prepared from the amino acids and potassium hydroxide and kept in a refrigerator until used. The pH of all solutions for the polarographic studies was measured by means of a Leeds and Northrup pH meter, assembly number 7661. To minimize errors in calculating the aspartate concentration from the pH, the pK_2 and pK_3 for aspartic acid was determined at the same ionic strength using carbonate-free sodium hydroxide. A simple method for determining the pK_2 and pK_3 of aspartic acid and equation for calculating the concentration of the aspartate ion will be given later in the paper. For alanine Keefer's value[7] of 9.86 for pK_2 was used.

Polarographic measurements were made using a Fisher Elecdropode. An H-type cell was used, with a saturated calomel electrode, in a thermostat kept at 25.0°. Oxygen was removed from the solutions in the cell with a stream of hydrogen and during each run an inert atmosphere of hydrogen was maintained over the solution. Methyl red (0.025%) and brom cresol green (0.12%) were used as a maximum suppressor. In the case of the aspartate, all solutions were made up to 5×10^{-4} M cupric nitrate and sufficient potassium nitrate was added to keep the ionic strength constant at 1.0. In the case of alanine, the composition of the solutions was as follows: 0.04 M potassium alaninate, 5×10^{-4} M cupric nitrate, 0.06 M potassium dihydrogen phosphate plus sodium hydroxide. The reversibility of the electrode reaction was tested for each analysis by determining the slopes of plots of log $i/(i_d - i)$ against $E_{d.e.}$, or by determining the values of $(E_{3/4} - E_{1/4})$,[8] where $E_{1/4}$ and $E_{3/4}$ are the values of $E_{d.e.}$ at $i = (3/4)i_d$ and $i = (1/4)i_d$, respectively. Corrections were made for residual current. Half-wave potentials were reproducible to ±3 mv.

Potentiometric studies were made using a Cenco Titration–pH meter, with a glass electrode which had been cleaned thoroughly with dilute acid, allowed to stand in distilled water for a number of hours, and then calibrated immediately before use by means of a potassium acid phthalate buf-

(1) Brother Edward Doody of the Congregation of Christian Brothers.

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(3) Presented at the 116th meeting of the American Chemical Society, Atlantic City, Sept., 1949.

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Exhibit I

and NMR spectrum), and 116 mg of 7 as a pale yellow, viscous oil: NMR (CDCl$_3$) δ 1.25 (m, 2 H), 1.68 (bs, 3 H), 1.82 (d, J = 0.9 Hz, 3 H), 2.17 (m, 2 H), and 7.04 and 7.11 (s's, 5 H each); mass spectrum M$^+$ 280.1257, (celed for $C_{18}H_{20}S$, 280.1254).

Conversion of 7 to *cis*-2-Phenylcyclopropanecarboxylic Acid (8). A solution of 80 mg of 7 in 2 ml of 1:1 dichloromethane–pyridine was cooled in a dry ice–acetone bath and was treated with a slight excess of ozone.[14] The reaction mixture was allowed to warm to 25 °C, poured into 25 ml of ether, and washed several times with 1 N hydrochloric acid. The extract was dried (MgSO$_4$) and the solvent was removed under reduced pressure, giving a yellow, viscous oil (ir 1702 cm^{-1}).

The residue was dissolved in 10 ml of 10% sodium hydroxide in 50% aqueous ethanol. The mixture was refluxed for 40 min, cooled, poured into 20 ml of water, and extracted with ether. The aqueous layer was acidified with hydrochloric acid and was extracted with two 10-ml portions of ether. The ether extract was dried (MgSO$_4$) and the solvent was removed under reduced pressure, leaving 15 mg of a tan solid whose NMR spectrum was identical with that of *cis*-2-phenylcyclopropanecarboxylic acid (8).[15] No peaks representing *trans*-2-phenylcyclopropanecarboxylic acid were present.

Reaction of 6 with Thiophenol. To a solution of 200 mg of 6 in 0.5 ml of hexadeuteriobenzene in an NMR tube was added 120 mg of thiophenol. The reaction mixture was thoroughly mixed and the rate of reaction was monitored with time by NMR. The reaction displayed an induction period of ~2 min, being essentially complete in 15 min at 39 °C. The resulting mixture was poured into 10 ml of ether and was extracted twice with 5-ml portions of 1 M sodium hydroxide, washed with water and saturated sodium chloride, and dried (MgSO$_4$). The solvent was removed under reduced pressure and the residue was distilled in a microstill at 115 °C (0.07 mm), giving a pale yellow, viscous oil: NMR of **9** (CDCl$_3$) δ 0.88 (dd, J = 5.0 and 8.1 Hz), 1.08 (dd, J = 2.3 and 8.1 Hz), 1.47 (s), 1.63 (dd, J = 2.3 and 5.0 Hz), 1.84 and 1.88 (broadened s's), 7.18; 10, δ 1.33 (s), 1.87 and 2.08 (broadened s's), 7.18 (the AMX double doublets are obscured by the more intense resonances of **9**); mass spectrum M$^+$ 294.1436 (calcd for $C_{18}H_{22}S$, 294.1442).

Reaction of 3 with Thiophenol. A solution of 430 mg (2.86 mmol) of 3 in 5 ml of benzene was added to 314 mg (2.86 mmol) of thiophenol dissolved in 5 ml of benzene. The reaction mixture was allowed to stand at room temperature for 3 h, at which time analysis by NMR indicated complete reaction. The benzene solution was washed with 15 ml of 10% sodium hydroxide and water, and was dried (MgSO$_4$). The benzene was removed under reduced pressure giving 656 mg of a pale yellow oil (11): bp ~50 °C (0.05 mm) in a molecular still; NMR (CDCl$_3$) δ 0.90 (s, 6 H), 0.98 (s, 6 H), 1.13 (m, 1 H), 1.83 (d, J = 1.6 Hz, 3 H), 1.92 (d, J = 2.2 Hz, 3 H), and 7.07 (m, 5 H); mass spectrum M$^+$ 260.1597 (calcd for $C_{17}H_{24}S$, 260.1608).

Ozonolysis of 11. A solution of 76 mg (0.30 mmol) of 11 in 1.75 ml of dichloromethane and 0.25 ml of pyridine[14] was cooled in a dry ice–acetone bath and ozone was bubbled through the solution for 15 s. The reaction mixture was allowed to warm to 25 °C and was analyzed directly by GLC on a Carbowax 20M column showing the presence of acetone by comparison of retention time with authentic materiel and admixture.

Registry No.—2, 4544-23-4; 3, 13303,30-5; 6, 40922-91-6; 7, 58873-30-6; 8, 939-89-9; 9, 58873-31-7; 11, 58873-32-8; thiophenol, 108-98-5.

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Trichloromethyl Chloroformate. Reaction with Amines, Amino Acids, and Amino Alcohols

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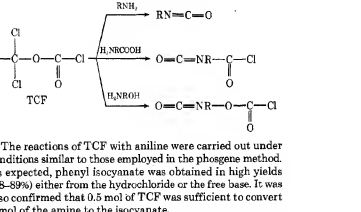
The title compound, trichloromethyl chloroformate (TCF), is of interest in that it is a potential substitute for phosgene, which presents a severe hazard in laboratory use because of its volatility and high toxicity. Although TCF is also toxic,[1] it is a dense liquid (bp 128 °C, d_4^{18} 1.65) with vapor pressure of only 10 mm at 20 °C. Thus TCF is more easily handled with safety, and seems to have significant advantages over phosgene.

Hentschel studied the decomposition of TCF and reactions with some organic compounds and found that phenyl isocyanate was formed by the action of TCF on 1,3-diphenylurea.[2] The reaction with alcohols to give carbonates has also been reported.[3] TCF was recently reported to be used as a substitute for phosgene in the preparation of *N*-carboxy-α-amino acid anhydrides; 1 mol of TCF provided the equivalent of 2 mol of phosgene in the NCA synthesis.[4]

To extend our knowledge of the reactivity of TCF, it was of interest to compare other reactions of TCF with those of phosgene. This paper describes the reaction of TCF with amines, amino acids, and amino alcohols to give the corresponding isocyanates, isocyanato acid chlorides, and isocyanato chloroformates.



The reactions of TCF with aniline were carried out under conditions similar to those employed in the phosgene method. As expected, phenyl isocyanate was obtained in high yields (78–89%) either from the hydrochloride or the free base. It was also confirmed that 0.5 mol of TCF was sufficient to convert 1 mol of the amine to the isocyanate.

Treatment of *p*-phenylenediamine hydrochloride with TCF in dioxane, on the other hand, gave only poor yields (23% or less) of the diisocyanate, even though the reaction was carried out under almost the same conditions used with phosgene. When the free base was used instead of the hydrochloride, the yield of the diisocyanate was improved to 47%. An attempted reaction of hexamethylenediamine hydrochloride with TCF in dioxane was unsuccessful and the hydrochloride was recovered. This result is presumably due to the high basicity of hexamethylenediamine compared to that of aromatic amines,

the more stable hydrochloride derived from the amine of higher basicity being less reactive to electrophilic attack.

The reactions of amino acids or amino alcohols with phosgene are interesting since they provide in one step molecules with two different functional groups, namely isocyanato acid chlorides or isocyanato chloroformates. The synthesis of 6-isocyanatohexanoyl chloride by the action of phosgene on the amino acid was reported to be attained only by using an additional reagent such as hydrogen chloride, thionyl chloride, or phosphorus pentachloride besides phosgene.[5] When TCF was used in this preparation, however, 6-isocyanatohexanoyl chloride was obtained in 73% yield without an additional reagent. TCF also reacted smoothly with 3-aminopropanoic acid, and in contrast to phosgene, 3-isocyanatopropanoyl chloride was obtained quantitatively (97%).

In contrast to the preparation of alkyl isocyanato acid chlorides, the TCF method with aromatic amino acids gave results similar to those with phosgene.[5] Treatment of *o*-aminobenzoic acid with TCF resulted in the formation of isatoic anhydride in a quantitative yield, as observed with phosgene. The reaction between *m*-aminobenzoic acid and TCF failed to give the corresponding isocyanato acid chloride, and only an unidentified white solid was obtained. It was confirmed that an additional reagent such as phosphorus pentachloride was necessary to prepare *o*-isocyanatobenzoyl chloride (85% yield) as in the phosgene method.[5]

Reactions of amino alcohols with TCF proceeded similarly to those with phosgene.[6] 3-Aminopropanol and 2-aminoethanol gave 3-isocyanatopropyl chloroformate and 2-isocyanatoethyl chloroformate, respectively, in 53 and 21% yields.

Thus it was found that TCF is far superior to phosgene in the alkyl isocyanato acid chlorides syntheses, but was comparable to phosgene in the preparations of phenyl isocyanate, aromatic isocyanato acid chlorides, and alkyl isocyanato chloroformates.

Experimental Section[7]

Phenyl Isocyanate. To a mixture of 12.95 g (0.1 mol) of aniline hydrochloride and 100 ml of dry dioxane was added 6.3 ml (10.4 g, 0.05 mol) of TCF. The mixture was heated at 60 °C; after 1.5 h of stirring, it became a clear solution. Heating was discontinued after 3.5 h and the solvent was removed under reduced pressure. The residue was distilled at 70–75.5 °C (36 mm) to give 10.8 g (88%) of phenyl isocyanate. It was redistilled almost quantitatively, bp 78–77 °C (39 mm) [lit.[8] 55–57 °C (16 mm)].

***p*-Phenylene Diisocyanate. A. From the Hydrochloride.** To 100 ml of dry dioxane were added 14.48 g (0.08 mol) of *p*-phenylenediamine hydrochloride and 51 ml (84.2 g, 0.4 mol) of TCF. The mixture was heated at reflux for 20 h. The unreacted hydrochloride was filtered off and the filtrate was evaporated under reduced pressure. The residual white, crystalline solid was sublimed under vacuum to give 3.0 g (23%) of *p*-phenylene diisocyanate. It was sublimed again at 85 °C (7 mm) to give colorless crystals, mp 92–94 °C [lit.[9] 94–96 °C].

B. From the Free Base. To a solution of 8.64 g (0.08 mol) of *p*-phenylenediamine in 100 ml of dry dioxane was added 20.5 ml (34.8 g, 0.16 mol) of TCF with stirring. Precipitation took place instantaneously. After refluxing the mixture for 20 h, the undissolved white solid was filtered off and the filtrate was evaporated. The residual solid gave 6.0 g (47%) of *p*-phenylene diisocyanate on sublimation.

3-Isocyanatopropanoyl Chloride. To 250 ml of dry dioxane were added 12.6 g (0.1 mol) of powdered 3-aminopropanoic acid hydrochloride and then 37.9 ml (62.6 g, 0.3 mol) of TCF with stirring. The mixture became a clear solution after heating at 55 °C for 4.5 h. The heating was continued for an additional 6.5 h and then the solvent was removed under reduced pressure. The residual oil was distilled to give 13.0 g (97%) of 3-isocyanatopropanoyl chloride, bp 77–80 °C (10 mm) [lit.[5] 91–91.5 °C (24.5 mm)].

6-Isocyanatohexanoyl chloride was synthesized by virtually the same procedure, bp 112–113 °C (5 mm) [lit.[5] 114 °C (6 mm)].

Reaction of *o*-Aminobenzoic Acid with TCF. A. Without PCl_5. A mixture of 10.0 g (0.073 mol) of *o*-aminobenzoic acid and 36.8 ml (60.7 g, 0.3 mol) of TCF in 150 ml of dry dioxane was refluxed for 6 h. The resulting clear solution was evaporated to give a white solid. It was recrystallized from tetrahydrofuran to give 10.0 g (92%) of isatoic anhydride, mp 241–243 °C dec (lit.[8] 242–243 °C dec).

B. With PCl_5. To a mixture of 10.0 g of *o*-aminobenzoic acid and 36.8 ml of TCF in 150 ml of dry dioxane was added 15.2 g (0.073 mol) of phosphorus pentachloride with stirring. Phosphorus pentachloride went into solution in 1 h. The solution was allowed to stand at room temperature overnight and then the solvent was removed under reduced pressure. The residue was distilled two times to give 11.2 g (85%) of *o*-isocyanatobenzoyl chloride, bp 108–109.5 °C (2 mm), mp 30–32 °C (lit.[5] 32 °C).

3-Isocyanatopropyl Chloroformate. To a solution of 48.4 ml (79.8 g, 0.4 mol) of TCF in 250 ml of dry dioxane was added 7.5 g (0.1 mol) of 3-aminopropanol dropwise over a period of 1 h with cooling in an ice bath. The mixture was stirred with cooling for 30 min and then left standing at room temperature overnight. The solution was evaporated under reduced pressure and the residue was distilled to give 10.2 g of distillate boiling at 65–105 °C (7 mm). Fractional redistillation afforded 0.5 g of a forerun boiling at 23–60 °C (1 mm) and 8.7 g (53%) of 3-isocyanatopropyl chloroformate boiling at 70–74.5 °C (1 mm) [lit.[6] 92 °C (1.5 mm)]. The forerun was considered to consist of mostly 3-chloropropyl isocyanate from its boiling range [lit.[6] 34 °C (1.5 mm)] and ir spectrum.

2-Isocyanatoethyl Chloroformate. 2-Aminoethanol (6.1 g, 0.1 mol) was treated with 24.2 ml (40 g, 0.2 mol) of TCF in 250 ml of dry dioxane at 55–60 °C for 6 h. Fractional distillation gave 0.7 g (3%) of 2-chloroethyl isocyanate boiling at 41.5–45 °C (13 mm) [lit.[6] 32–36 °C (13 mm)], 3.2 g (21%) of 2-isocyanatoethyl chloroformate boiling at 89.5–90 °C (14 mm) [lit.[6] 86–87 °C (13 mm)], and 1 g (6%) of 2-oxazolidone boiling at 160–165 °C (2 mm), mp 86–88 °C (lit.[6] 89 °C).

Acknowledgment. The authors wish to express their appreciation to Hodogaya Kagaku Kogyo Co. for a gift of TCF.

Registry No.—Phenyl isocyanate, 103-71-9; aniline hydrochloride, 142-04-1; *p*-phenylene diisocyanate, 104-49-4; *p*-phenylenediamine hydrochloride, 624-18-0; *p*-phenylenediamine, 106-50-3; 3-isocyanatopropanoyl chloride, 3729-19-9; 3-aminopropanoic acid hydrochloride, 6087-90-5; 6-isocyanatohexanoyl chloride, 3729-18-8; *o*-aminobenzoic acid, 118-92-3; isatoic anhydride, 118-48-9; *o*-isocyanatobenzoyl chloride, 5100-23-2; 3-aminopropanol, 156-87-6; 3-isocyanatopropyl chloroformate, 13107-90-9; 3-chloropropyl isocyanate, 13010-19-0; 2-aminoethanol, 141-43-5; 2-chloroethyl isocyanate, 1943-83-5; 2-isocyanatoethyl chloroformate, 13107-89-6; 2-oxazolidone, 497-25-6; TCF, 23213-83-4; PCl_5, 10026-13-8.

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An Electron Spin Resonance Study of the Radical Anion of 7,8-Dimethylene-1,3,5-cyclooctatriene

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Received February 20, 1976

Several examples of pericyclic reactions in radical anions are known where the stereochemistry is the same as that of the excited state of the neutral molecule.[1] If these reactions are concerted, the parallel mode of reaction of the radical anions with the excited states is predicted by the highest occupied molecular orbital (HOMO) method.[2] Bauld and Cessac[3] have recently noted that the butadiene–cyclobutene

Exhibit J

Available Upon Request

Exhibit L

Use of the Kaiser Oxime Resin in the Solid-Phase Synthesis of 3-Aminobenzisoxazoles

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Received January 20, 1999

In connection with our efforts to identify ligands for a variety of biological targets, we have been interested in the synthesis of 3-aminobenzisoxazoles.[1] To take advantage of parallel synthesis techniques,[2] we sought to develop a method for the solid-phase synthesis of these interesting heterocycles. Two methods for the preparation of 3-aminobenzisoxazoles have been reported. Palermo describes a one-pot procedure involving an S_NAr reaction of an activated *ortho*-halobenzonitrile with hydroxamate ion.[3] Alternatively, Shutske uses a two-step process involving acetone oxime addition to 2-fluorobenzonitrile followed by a subsequent acid-mediated cyclization (Figure 1).[4] We found the two-step approach using polymer-bound oxime particularly attractive, because in theory, one could use the aryl oxime intermediate as a linker. Acid-mediated cyclization would then effect a cyclorelease[5] from the resin, leaving no apparent residual functionality from linkage to the solid phase.

To implement this strategy, we obtained the oxime-containing resin **1** (Scheme 1), which was originally developed by Kaiser and Degrado for solid-phase peptide synthesis[6] and subsequently used in the synthesis of cyclic peptides,[7] ureas,[8] and hydroxamic acids.[9] An attractive feature of the Kaiser resin is the incorporation of the nitro substituent on the benzophenone oxime, which enhances the stability of acylated intermediates to anhydrous acid. We hypothesized that aryl oxime adducts (**3**) would be at least as stable as acylated oxime intermediates, providing chemical compatibility of this linkage for library generation. Therefore, we sought to demonstrate that the Kaiser resin could serve as a nucleophile in S_NAr reactions and that the resulting aryl oxime could be used as a linking group in the solid-phase synthesis of 3-aminobenzisoxazoles.

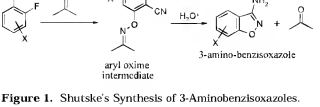
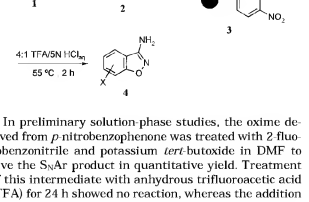


Figure 1. Shutske's Synthesis of 3-Aminobenzisoxazoles.

Scheme 1. Reaction of 2-Fluorobenzonitriles with Oxime Resin 1 Followed by Cyclitive Removal



In preliminary solution-phase studies, the oxime derived from *p*-nitrobenzophenone was treated with 2-fluorobenzonitrile and potassium *tert*-butoxide in DMF to give the S_NAr product in quantitative yield. Treatment of this intermediate with anhydrous trifluoroacetic acid (TFA) for 24 h showed no reaction, whereas the addition of 20% water gave 50% conversion to 3-aminobenzisoxazole (**4a**) after 24 h.

Although these conditions for acid promoted cyclization are not optimized, the stability of the intermediate to anhydrous acid and the lability under aqueous conditions suggested that the desired transformations could be achieved on resin. Therefore, we moved on to optimize the conditions for these reactions on the solid phase, where we examined the influence of both steric and electronic factors on the loading and cyclitive removal steps.

The effect of solvent on the loading reaction was explored in the case of 4-bromo-2-fluorobenzonitrile (**2e**, Table 1), with DMF, *N*-methylpyrrolidinone, DMSO, and THF giving 58%, 58%, 52%, and 90% yield, respectively.[10] Although DMF is the solvent of choice for the solution phase reaction, THF is preferred on solid phase, presumably as a result of its superior resin-swelling ability. Column 3 of Table 1 gives loading yields for a variety of 2-fluorobenzonitriles. The presence of an electron-withdrawing group facilitates loading at room temperature. These electron-poor aryl fluorides can also be loaded equally well at 55 °C, except in the case of **2f**, which

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(10) The % loading determinations of entries **a**−**h** in Table 1 are an average of 3 experiments and were calculated on the basis of weight difference. IR spectra for these loaded resins showed the requisite nitrile absorptions from 2225 to 2235 cm^{-1}. In addition, % loading was also calculated for entries **a** and **c**−**f** on the basis of nitrogen combustion analysis (n = 12) and were found to be in close correspondence with yield determinations based on weight. See ref 8 for discussion of weight versus elemental analysis for yield determination.

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Table 1. Loading Yields of Oxime Adducts 3; HPLC Purity and % Isolated Yields of 3-Aminobenzisoxazoles 4

entry	2 (X =)	% yield of 3[a]	purity of crude 4[b]	% isolated yield of 4[c]
a	H	64	>96	76
b	3-CF_3	83	>96	62
c	4-MeO	80	>96	85
d	4-CF_3	90	>96	86
e	4-Br	90	>96	78
f	4-CN	83	84[d]	55
g	5-NO_2	95	79	70
h	6-CF_3	69	>96	75

[a] Determined by resin weight difference (av of 3 experiments, see ref 8). [b] As determined by reverse-phase HPLC. [c] Compounds **4a**–**h** were purified by silica gel chomatography. Yields based on loading yield of **3**. [d] Major impurity was 7-carboxy-3-aminobenzisoxazole (hydrolysis of the nitrile group).

required room temperature for optimal loading (83% vs 69%). Substrates bearing electronically neutral or donating groups require heating to 55 °C in order to load in a reasonable time frame (12 h). As shown by entry **b**, the loading reaction is not sensitive to steric hindrance around the site of nucleophilic substitution.

We observed facile cyclitive removal of compounds **4a**–**c** and **4h**, with reasonable crude purity and high isolated yield using 99:1 TFA/H_2O at 55 °C for 12 h. However, application of the same conditions to **3d**–**f** and **3g** gave much lower isolated yields (crude purity of 60–80%) after 12 h and required several days to reach completion. After we explored a variety of acidic conditions, 4:1 TFA/aqueous 5 N HCl was found to give markedly better results at much shorter reaction times. Column 5 (Table 1) gives the isolated yield of benzisoxazoles **4a**–**h** using these conditions, based on percent loading. As shown in column 4 (Table 1), crude cyclization products (**4**) were generally quite pure. A noticeable exception was entry **f** (Table 1). When **3f** was subjected to cyclitive removal conditions for 2 h, the crude purity of the corresponding product (**4f**) was 72% (isolated yield 68%), with the major impurity arising from hydrolysis of the 4-nitrile group. This purity can be improved to 84% by reducing the cyclitive removal time to 1 h with a modest drop in yield.

Having identified acceptable conditions for loading and cyclitive removal of a variety of substrates, we moved on to confirm the compatibility of the aryl oxime linker with the anhydrous acidic conditions suggested by the solution-phase model study. Thus, the BOC-protected amine **5** (Scheme 2) was prepared and loaded onto the Kaiser resin in 53% yield. The resin was then treated with 25% TFA/CH_2Cl_2 for 2 h, rinsed, and vacuum-dried to give **7** (quantitative) as determined by resin weight difference. The resin washing solutions from this reaction were concentrated and provided no UV-active material by TLC. Resin **7** was then treated with a variety of electrophiles

Scheme 2. On-Resin BOC Removal Followed by Treatment with Electrophiles

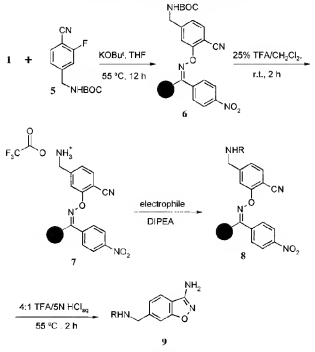


Table 2. Product Yield and Purity of 9

entry	electrophile	R	purity of crude 9[a]	% isolated yield of 9[b]
a	acetic anhydride		95	79
b	4-bromo-benzoyl chloride		93	70
c	4-bromo-phenyliso-cyanate		93	72
d	4-bromo-phenylthio-isocyanate		94	70

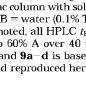
[a] As determined by reverse-phase HPLC. [b] Compounds **9a**–**d** were purified by silica gel chomatography. Yields based on loading yield of **5**.

(as shown in entries **a**–**d** in Table 2) to give **8**. Table 2 gives the crude purities and isolated yields of **9a**–**d** after cyclitive removal.

In conclusion, we have developed an efficient method for the solid-phase synthesis of 3-aminobenzisoxazoles. Our approach involves the first application of the Kaiser oxime resin to S_NAr reactions and can be used successfully with 2-fluorobenzonitriles containing a variety of electron-withdrawing/donating groups. The intermediate aryl oxime linkage is stable to anhydrous acid and can support BOC-deprotection and amidation reactions, holding promise for compatibility with a broader variety of organic transformations. Progress toward the expansion of this methodology, as well as its application to the synthesis of directed libraries, will be reported in due course.

Experimental Section

General. Except for compound **2c** and **2f**, all reagents used were obtained from commercial sources. Reagents were used without further purification. Kaiser oxime resin **1** was purchased from Novabiochem with a loading capacity of 1.07 mmol/g. All NMR spectra (400 MHz) were recorded on a Varian Gemini-400 spectrometer. Mass spectra were obtained with either ESI or FAB as the ionization method. All purifications were carried out by radial chromatography (Chromatotron model 8924, Harrison Research) using 1 mm silica gel plates (Analtech). Crude purities were estimated from integrated peak areas of HPLC chromatographs with the UV detector monitoring at λ = 215 nm. Analytical HPLC setup: C_{18} Vydac column with solvent gradient A = acetonitrile (0.1% TFA) and B = water (0.1% TFA) at 1 mL/min flow rate. Unless otherwise noted, all HPLC t_R are given for an eluent gradient of 10% A to 60% A over 40 min. The nomenclature for compounds **4a**−**h** and **9a**−**d** is based on the numbering system given in ref 4a and reproduced here:



General Synthesis of 3-Amino-1,2-benzisoxazoles 4a−**h. Formation of 3-Amino-1,2-benzisoxazole (4a).** To *p*-nitrobenzophenone oxime polystyrene (Kaiser) resin (500 mg, 1.07 mmol/g, 0.54 mmol) in a tared 25 mL Kontes microfilter funnel was added THF (7 mL) and potassium *tert*-butoxide (640 μL, 1 M in THF, 0.642 mmol). After being shaken by hand for several minutes, the resin turned a deep purple color. To this suspension was added 2 fluorobenzonitrile (1.07 mmol, 214 mg), and the reaction vessel was rotated at 55 °C in a Robbins oven for 12 h, removed from the oven, and allowed to cool for 1 h. The resin was then rinsed with 2 × 5 mL of CH_2Cl_2, 2 × 5 mL of 5% TFA/CH_2Cl_2, 2 × 5 mL of 2-propanol, and 4 × 5 mL of MeOH and then dried in a 35 °C vacuum oven for 12 h to give a Δwt of 36.5 mg (66% loading yield). TFA (4 mL) and 5 N HCl_{aq} (1 mL) were then added to the resin, followed by rotation for 2 h in a 55 °C oven. The TFA/HCl_{aq} was collected, and the resin was rinsed with 2 × 5 mL of CH_2Cl_2. These washings were combined and concentrated in vacuo to give the crude product **4a** (>96% purity by reverse-phase HPLC), which was purified by radial chromatography using a 2 mm plate and eluting with 25% EtOAc/hexanes. Concentration of the product containing fractions gave pure **4a** (35 mg; 2 step yield, 49% based on theoretical equivalents of oxime in the starting resin or 74% based on loading yield). **3-Amino-1,2-benzisoxazole (4a).** HPLC t_R = 12.3 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (d, J = 7.7 Hz, 1H), 7.48 (t, J = 8.4 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.21 (t, J = 7.0 Hz, 1H), 6.38 (bs, 2H). MS (ESI) m/z 135 (M + H)$^+$. Anal. Calcd for $C_7H_6N_2O$: C, 62.68; H, 4.51; N, 20.88. Found: C, 62.61; H, 4.46; N, 20.83.

7-Trifluormethyl-3-amino-1,2-benzisoxazole (4b). HPLC t_R = 21.9 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (d, J = 8.4 Hz, 1H), 7.71 (t, J = 8.4 Hz, 1H), 7.64 (d, J = 7.2 Hz, 1H), 5.82 (bs, 2H). MS (ESI) m/z 203 (M + H)$^+$. Anal. Calcd for $C_8H_5F_3N_2O$: C, 47.54; H, 2.49; N, 13.86; F, 28.20. Found: C, 47.71; H, 2.60; N, 13.85; F, 28.37.

6-Methoxy-3-amino-1,2-benzisoxazole (4c). HPLC t_R = 14.2 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (d, J = 8.4 Hz, 1H), 6.95 (d, J = 2.4 Hz, 1H), 6.80 (dd, J = 8.4, 2.4 Hz, 1H), 6.23 (bs, 2H). MS (ESI) m/z 165 (M + H)$^+$. Anal. Calcd for $C_8H_8N_2O_2$: C, 58.53; H, 4.91; N, 17.06. Found: C, 58.78; H, 4.99; N, 17.10.

6-Trifluormethyl-3-amino-1,2-benzisoxazole (4d). HPLC t_R = 24.6 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (d, J = 8.4 Hz, 1H), 7.90 (s, 1H), 7.58 (d, J = 8.4 Hz, 1H), 6.64 (bs, 2H). MS (ESI) m/z 203 (M + H)$^+$. Anal. Calcd for $C_8H_5F_3N_2O$: C, 47.54; H, 2.49; N, 13.86; F, 28.20. Found: C, 47.47; H, 2.50; N, 13.59; F, 27.96.

6-Bromo-3-amino-1,2-benzisoxazole (4e). HPLC t_R = 22.6 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.76 (s, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 6.44 (bs, 2H). MS (ESI) m/z 213 [^{79}Br, (M + H)$^+$], 215 [^{81}Br, (M + H)$^+$]. Anal. Calcd for $C_7H_5BrN_2O$: C, 39.47; H, 2.37; N, 13.15; Br, 37.51. Found: C, 39.30; H, 2.26; N, 13.09; Br, 37.58.

6-Cyano-3-amino-1,2-benzisoxazole (4f). HPLC t_R (eluent gradient 5% A to 40% A over 40 min) 17.2 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H), 6.67 (bs, 2H). MS (ESI) m/z 160 (M + H)$^+$. Anal. Calcd for $C_8H_5N_3O_2$: C, 60.38; H, 3.17; N, 26.40. Found: C, 60.17; H, 3.46; N, 24.82.

5-Nitro-3-amino-1,2-benzisoxazole (4g). HPLC t_R = 15.9 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (d, J = 2.8 Hz, 1H), 8.36 (dd, J = 9.2, 2.8 Hz, 1H), 7.65 (d, J = 9.2 Hz, 1H), 6.82 (bs, 2H). MS (ESI) m/z 180 (M + H)$^+$. Anal. Calcd for $C_7H_5N_3O_3$: C, 46.94; H, 2.81; N, 23.46. Found: C, 46.85; H, 2.80; N, 23.26.

4-Trifluoromethyl-3-amino-1,2-benzisoxazole (4h). HPLC t_R = 21.5 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (d, J = 8.4 Hz, 1H), 7.71 (t, J = 8.4 Hz, 1H), 7.64 (d, J = 7.2 Hz, 1H), 5.81 (bs, 2H). MS (ESI) m/z 203 (M + H)$^+$. Anal. Calcd for $C_8H_5F_3N_2O$: C, 47.54; H, 2.49; N, 13.86; F, 28.20. Found: C, 47.32; H, 2.62; N, 13.92; F, 27.96.

General Synthesis of 3-Aminobenzisoxazoles 9a−**d. Formation of 6-(Acetylaminomethyl)-3-amino-1,2-benzisoxazole (9a).** To *p*-nitrobenzophenone oxime polystyrene (Kaiser) resin (500 mg, 1.07 mmol/g, 0.54 mmol) in a tared 25 mL Kontes microfilter funnel was added THF (7 mL) and potassium *tert*-butoxide (640 μL, 1 M in THF, 0.642 mmol). After being shaken by hand for several minutes, the resin turned a deep purple color. To this suspension was added 4-(*tert*-butoxycarbonylaminomethyl) 2 fluorobenzonitrile (1.07 mmol, 268 mg). The reaction vessel was rotated at 55 °C in a Robbins oven for 12 h and allowed to cool for 1 h. The resin was then rinsed with 2 × 5 mL of CH_2Cl_2, 2 × 5 mL of 5% TFA/CH_2Cl_2, 2 × 5 mL of 2-propanol, and 4 × 5 mL of MeOH. The resin was dried in a 35 °C vacuum oven for 12 h to give a Δwt of 65.0 mg (53% loading yield). To effect BOC-deprotection, the resin was then suspended in 25% TFA/CH_2Cl_2 (7 mL) and rotated for 2 h. The resin was again rinsed with 2 × 5 mL of CH_2Cl_2, 2 × 5 mL of 2-propanol, and 4 × 5 mL of MeOH. The resin was then suspended in DMF (7 mL), followed by addition of acetic anhydride (505 μL, 6.42 mmol) and *N*,*N*-diisopropylethylamine (930 μL, 6.42 mmol). After the reaction vessel rotated for another 12 h, the resin was rinsed with 2 × 5 mL of CH_2Cl_2, 2 × 5 mL of 2-propanol, and 4 × 5 mL of MeOH. TFA (4 mL) and 5 N HCl_{aq} (1 mL) were then added to the resin, and the vessel was rotated for 2 h in a 55 °C oven. The TFA/HCl_{aq} was collected, and the resin was rinsed with 2 × 5 mL of CH_2Cl_2. These washings were combined and concentrated in vacuo to give the crude product **9a** (95% purity by reverse-phase HPLC), which was purified by radial chromatography on a 2 mm plate, eluting with 50% EtOAc/hexanes. Concentration of the product containing fractions gave pure **9a** (45 mg; 4 step yield, 41% based on 1.07 mmol/g given by the manufacturer or 79% yield based on loading yield).

6-(Acetylaminomethyl)-3-amino-1,2-benzisoxazole (9a). HPLC t_R = 10.5 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (bs, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.24 (s, 1H), 7.10 (d, J = 8.0 Hz, 1H), 6.32 (bs, 1H), 4.32 (d, J = 6.4 Hz, 2H), 1.86 (s, 3H). MS (ESI) m/z 206 (M + H)$^+$. Anal. Calcd for $C_{10}H_{11}N_3O_2$: C, 58.53; H, 5.40; N,20.48. Found: C, 57.77; H, 5.45; N, 19.92.

6-[(*p*-Bromophenylcarbonyl)aminomethyl]-3-amino-1,2-benzisoxazole (9b). HPLC t_R = 21.5 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (bs, 1H), 7.82 (d, J = 8.4 Hz, 2H), 7.72 (d, J = 8.1 Hz, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.31 (s, 1H), 7.18 (d, J = 8.1 Hz, 1H), 6.34 (bs, 2H), 4.55 (d, J = 5.9 Hz, 2H). MS (ESI) m/z 346 [^{79}Br, (M + H)$^+$], 348 [^{81}Br, (M + H)$^+$]. Anal. Calcd for $C_{15}H_{12}BrN_3O_2$: C, 52.05; H, 3.49; N, 12.14. Found: C, 51.80; H, 3.54; N, 11.67.

6-[(*p*-Bromophenylaminocarbonyl)aminomethyl]-3-amino-1,2-benzisoxazole (9c). HPLC t_R = 28.2 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (bs, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.35 (s, 4H), 7.29 (s, 1H), 7.15 (d, J = 8.4 Hz, 1H), 6.76 (t, J = 6.2 Hz, 1H), 6.33 (bs, 2H), 4.38 (d, J = 6.2 Hz, 2H). MS (ESI) m/z 361 [^{79}Br, (M + H)$^+$], 363 [^{81}Br, (M + H)$^+$]. Anal. Calcd for $C_{15}H_{13}BrN_4O_2$: C, 49.88; H, 3.63; N, 15.51. Found: C, 49.78; H, 3.93; N, 15.10.

6-[(*p*-Bromophenylaminothiocarbonyl)aminomethyl]-3-amino-1,2-benzisoxazole (9d). HPLC t_R = 30.5 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (bs, 1H), 8.32 (bs, 1H), 7.72 (d, J = 8.1 Hz, 1H), 7.47 (d, J = 8.8 Hz, 2H), 7.39 (d, J = 8.8 Hz, 2H), 7.32 (s, 1H), 7.19 (d, J = 8.1 Hz, 1H), 6.34 (bs, 2H), 4.81 (s, J = 5.5 Hz, 2H). MS (FAB) m/z 377 [^{79}Br, (M + H)$^+$], 379 [^{81}Br, (M + H)$^+$]. Anal. Calcd for $C_{15}H_{13}BrN_4OS$: C, 47.76; H, 3.47; N, 14.85. Found: C, 47.79; H, 3.29; N, 14.68.

Acknowledgment. The authors would like to thank Dr. Steven W. Kaldor for many helpful discussions.

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Synthesis of *Meta*-Substituted Aniline Derivatives by Nucleophilic Substitution

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Abstract: Substitution by amines of fluorobenzenes containing a *meta*-substituted electron withdrawing group (EWG), in DMSO at 100 °C over 60 h gave *meta*-substituted aniline derivatives in isolated yields of up to 98%. The scope of the reaction is explored in terms of reaction conditions and substrates. It is postulated that facile *meta*-substitutions are facilitated through field stabilisation of the intermediate anion by EWG substituents.

Keywords : Amination, Aryl halides, Substituent effects

Introduction

Anilines are very important synthetic intermediates and are used widely in the preparation of pharmaceuticals, dyes, pesticides and heterocyclic compounds. It is therefore important that methods exist for the efficient synthesis of novel anilines on laboratory to manufacturing scales, and that new methods are developed for the synthesis of less accessible derivatives. Historically,[1] aniline synthesis was carried out mainly by electrophilic aromatic substitution in two steps (Scheme 1): nitration or nitrosation, followed by catalytic hydrogenation or reduction with a metal salt. These procedures have found large scale use, but have some limitations, such as the use of strong acidic or oxidizing reaction conditions, and *ortho*/*para* mixtures may be obtained. Aromatic nucleophilic substitution[2] has the potential advantage of being a single reaction step, but until recently was restricted[2] in synthetic use to substrates containing an electron withdrawing group (EWG), and also in most cases to *ortho*/*para* substitution patterns, with *meta*-substitution giving poor synthetic yields. In recent years however, the nucleophilic substitution approach has been much developed by the discovery[3, 4, 5] of metal-catalyzed carbon-nitrogen bond forming reactions, which do not require the presence of EWGs, and the continued development of the existing S_NAr substitution process. This paper describes the synthesis of *meta*-substituted anilines in high yields under modified S_NAr reaction conditions, and compares these yields with the known palladium catalyzed methods. This work has appeared[6] in part as a preliminary communication.

We required an efficient synthesis of the morpholine-substituted benzoic acids **1a-c**, which preferably

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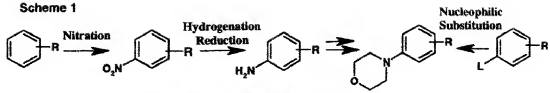
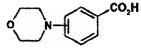
Scheme 1



R = variety of groups; L = leaving group

could be operated cost effectively on larger scales, and did not require palladium catalysts or the presence of phosphine ligands. In this situation there is an even greater advantage in employing nucleophilic substitution



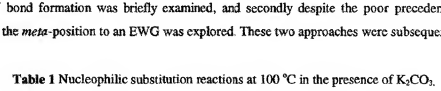
1a, 2-substituted
1b, 3-substituted
1c, 4-substituted

with morpholine, rather than electrophilic substitution, because with electrophilic substitution the amine substituent obtained after the reduction step requires elaboration to the morpholine ring system, which is known[7] to be a lengthy process involving several synthetic steps. The morpholine-substituted acids **1a-c** were conveniently sought *via* the synthesis of their corresponding esters **2a-c** and nitriles **3a-c** (Table 1).

Results and Discussion

Reaction of morpholine (Table1) with ethyl fluorobenzoates or benzonitriles at 100 °C in DMSO solvent for 18 h and with anhydrous K_2CO_3 present, gave adequate yields of the *ortho*- and *para*-substituted derivatives **2a**, **2c**, **3a**, and **3c**, but in line with expectations[1] it failed to afford any *meta*-substituted **2b**, and gave only a poor 14% yield of **3b**. Two approaches were followed in order to seek a more efficient *meta*-substitution: palladium catalysed C-N bond formation was briefly examined, and secondly despite the poor precedent,[1] nucleophilic substitution in the *meta*-position to an EWG was explored. These two approaches were subsequently compared.

Table 1 Nucleophilic substitution reactions at 100 °C in the presence of K_2CO_3.



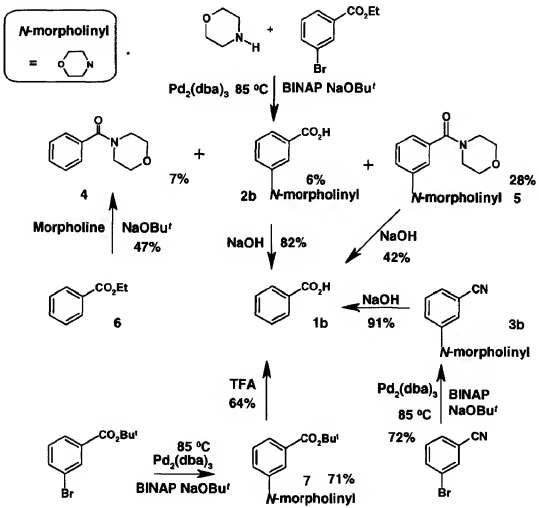
2a-c, 3a-c

Substituent	R = CO_2Et	Yield %	R = CN	Yield %
2-F	**2a**	28	**3a**	82
3-F	**2b**	0	**3b**	14
4-F	**2c**	47	**3c**	94

Palladium catalyzed substitution

The palladium catalyzed reaction (Scheme 2) was employed using the optimal reported[6] reaction conditions: with bromide as the leaving group, NaOBu^t as the base, tris(dibenzylideneacetone) dipalladium(0) as the catalyst, and BINAP as the ligand. *N*-benzoylmorpholine **4** and the desired product **2b** were obtained in very poor yield, but the major product was the morpholinobenzamide **5**. Both the amide **5** and the morpholine **2b** were hydrolysed with 5M NaOH to give good yields of **1b**.

Scheme 2



To ascertain which reagent had led to the formation of the unwanted morpholinoamide **5**, morpholine was allowed to react with ethyl benzoate **6** (Scheme 2) at 85 °C in toluene for 18 h. In the presence of morpholine alone, none of the ethyl benzoate was converted to the morpholinobenzamide **4**. When NaOBu^t was added however, a 47% yield of **4** was obtained, which increased to only 56% in a reaction containing the

palladium catalyst as well. On the basis of these experiments, amide formation was attributed to the presence of butoxide ion. When the weaker and more soluble base caesium carbonate was used in place of $NaOBu^t$ in a reaction with ethyl 3-bromobenzoate under the same palladium catalyzed reaction conditions[8] as above, an improved 38% yield of **2b** was obtained, without amide formation occurring.

Morpholine amide formation was more successfully avoided under palladium catalysis[8] with $NaOBu^t$, by using the corresponding *t*-butyl ester or by use of the analogous nitrile. Thus *t*-butyl 3-bromobenzoate gave the morpholine derivative **7**, (71%), which hydrolysed in TFA to **1b** (64%). Secondly, reaction with 3-bromobenzonitrile afforded **3b** (72%), which hydrolysed readily with NaOH in *n*-BuOH to give **1b** (91%).

Substitutions without palladium catalysis

These modified palladium catalyzed reactions described above gave the *meta*-substituted products in good yields, and the amide formation issue had been successfully addressed. Nevertheless, the nucleophilic substitution reactions were re-examined to discover if reaction conditions could be found for larger scale experiments, that did not involve palladium reagents or BINAP. Some support for attempting this approach came from the observation that the stronger electron withdrawing CN-group gave a 14% yield of **3b** (Table 1) in replacement reactions with morpholine, and an early report[9] of a mechanistic study, which showed that *meta*-nucleophilic substitutions could proceed when there were two strong EWGs *meta*-disposed to the leaving group.

Exploration of the reaction conditions for *meta*-substitution was first restricted to the synthesis of **3b**, using 3-fluorobenzonitrile and morpholine as substrates, in K_2CO_3/DMSO at 100 °C during 18 h. Extending the reaction time from 18 to 60 h increased the yield of **3b**, to 29%, and in an attempt to obtain a further yield improvement, homogeneous reaction conditions were explored with the K_2CO_3 replaced as base by an equivalent amount of morpholine (*ie*. a total of 5.5 equivalents); here the yield of **3b** was increased to 62%. The structure of this material was carefully considered, because of the unexpectedly high yield; was it correct, and were other regioisomers present? The ^{1}H-NMR spectroscopic data for **3b** indicated that only the *meta*-product had been formed, and this was supported by TLC comparisons with authentic samples of **3a** and **3c**. Examination of the proton splitting patterns and chemical shifts obtained from the *ortho*-, and *para*-morpholinobenzonitriles **3a, 3c**, also showed there were no signals in the **3b** 60h/DMSO reaction product spectrum consistent with the presence of *ortho*- or *para*-substitution. The ^{13}C-NMR spectrum of **3b** confirmed that it was *meta*-substituted with no *ortho* or *para* products present, and the spectra were consistent with carbon chemical shift predictions.[10]

Neither increasing the reaction time to 90 h (58%), increasing the reaction temperature to 130 °C (60%), changing the solvent to dimethylacetamide (18%), NMP (17%) or 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU, 51%), employing DABCO catalysis (55%), nor increasing the amount of morpholine to an excess of 15 equivalents (46%), could significantly increase the 62% yield of **3b**. These experiments indicated that the substitution conditions employing a 5.5 equivalent excess of morpholine in DMSO for 60 h

were optimum, and they gave reproducible yields of **3b** even on a 150 g scale. These reaction conditions were used in all of the investigations concerning: the leaving group, substrate and nucleophile variation.

Leaving group variation

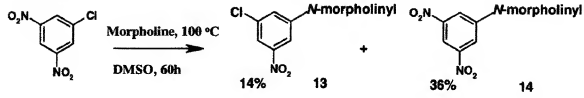
Halogen, nitro and triflate leaving groups were examined in compounds **8**-**12** with morpholine as the nucleophile, and reaction did not proceed except for the triflate **10**, (48%) indicating that with a single EWG

8, X = Cl
9, X = Br

10, X = OTf
11, X = NO_2
12, X = Cl

substituent, fluoride and triflate were the only viable leaving groups. It was anticipated that a second *meta*-substituted EWG would give sufficient electron withdrawal for amination to proceed with the poorer leaving groups, and this was the case (Scheme 3). 3,5-Dinitrochlorobenzene afforded a mixture with two isolable

Scheme 3

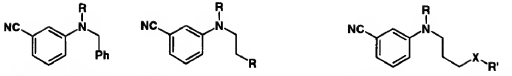


products: **13**, (14%) resulting from replacement of a nitro substituent, and **14** (36%) the major product resulting from chloride substitution. In addition 4% of starting halide was recovered, with the remainder being decomposed material.

Nature of the amine nucleophile

Good yields were obtained when 3-fluorobenzonitrile was allowed to react with cyclic secondary amines: morpholine (62%, **3b**), piperidine (68%, **15**), *N*-methylpiperazine (69%, **16**), and pyrrolidine (64%, sealed tube **17**), but with the benzylamine **18a** a poor 22% yield was obtained and its more hindered homologue failed to afford **18b**. No reaction was observed with 3-fluorobenzonitrile and cyclohexylamine or *t*-butylamine indicating that branching on the α-carbon of the alkylamine caused a steric effect, but *n*-butylamine afforded a low 10% yield of **19a**. Higher isolated yields were obtained when the amine component contained a hetero-substituent, as in **19b**-**20c** (31-58%).

Reaction of 3-fluorobenzonitrile with 2 equivalents of piperazine (Scheme 4) gave the *N*-mono-substituted product **21** in 57% yield, with none of the disubstituted **22**. With 5.5 equivalents of piperazine this



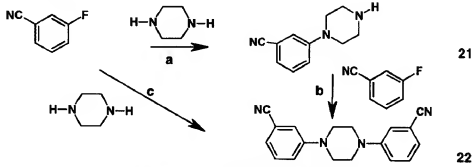
18a R = Me, **22%**
18b R = Et, **0%**

19a R = H, R' = Et **10%**
19b R = Me, R' = CH_2NMe_2 **58%**
19c R = H, R' = NMe2 **34%**
19d R = H, R' = CH_2NMe_2 **36%**
19e R = H, R' = $(CH_2)_2NMe_2$ **39%**

20a X = O, R = H, R' = Me **42%**
20b X = O, R = H, R' = ^nBu **38%**
20c X = S, R = H, R' = Me **31%**

yield increased to 68%, with still none (by TLC/isolation) of **22** being present in the reaction product. Attempts to prepare **22** directly from piperazine and 2 equivalents of 3-fluorobenzonitrile in the presence of 3 equivalents of K_2CO_3 afforded mixtures containing only traces of **22**, but a 17% yield was obtained when equimolar amounts of 3-fluorobenzonitrile and **21**, were allowed to react with 3 equivalents of K_2CO_3.

Scheme 4

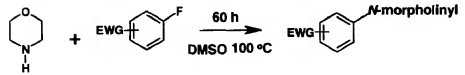


Yields : a = 57 and 68%; b = 17%; c = 0%

Fluoro-substituted substrates

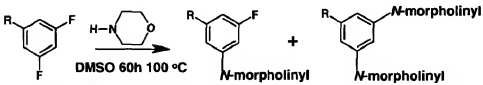
The extent to which fluorobenzenes (Table 2) reacted with morpholine in DMSO at 100 °C depended principally on the ring EWG, as yields of over 70% were obtained with substituents having Hammett σ-*meta* substituent values[11] >0.7, whereas values < 0.4 gave yields of <10%. The nitro and cyano-substituted compounds **31-34** gave poor yields with the electron donating methyl group being present as a substituent. When two fluoro leaving groups were present mixtures of mono- and di-substituted products were obtained (Table 3) with the mono-substituted product predominating. In a ^{1}H-NMR experiment in DMSO-d_6 at 100 °C, 3,5-difluoronitrobenzene was completely converted to **37** after 1h and the disubstituted **38** was only detectable after 3h. This experiment indicated that formation of the disubstituted products was a two step process. Reaction of 3,5-difluoronitrobenzene and morpholine at room temperature over 60h gave a 91% isolated yield of **37** and none of **38**. In **39** the ester EWG was not sufficiently electron withdrawing for reaction to di-substituted

Table 2 Substrate variation in reactions with morpholine at 100 °C during 60 h



Compd	EWG	% Yield	Compd	EWG	% Yield
23	3-NO_2	98	**29**	H	0
24	3-CO_2Et, 5-CF_3	74	**30**	F	8
25	3-CN, 5-CF_3	97	**31**	2-Me, 5-NO_2	1
26	3-CF_3	19	**32**	2-Me, 3-NO_2	0
27	3-CO_2Et	3	**33**	3-NO_2, 4-Me	20
28	3-CO_2H	0	**34**	3-CN, 4-Me	4

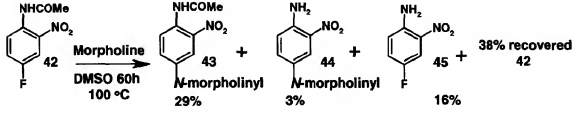
Table 3 Substitution of 3, 5-difluoro-compounds



R	Compd	% yield	Compd	% yield
		Mono-substitution		Di-substitution
CN	**35**	62	**36**	37
NO_2	**37**	78	**38**	16
CO_2Et	**39**	77	-	-

products. A subsequent experiment where **35**, and **37** were allowed to react with morpholine under the same standard reaction conditions, gave poor yields of **36** (8%) and **38** (19%). Reaction of ethyl 3,5-difluorobenzoate (in a sealed tube, but otherwise using the standard DMSO/60h/100 °C reaction conditions)

Scheme 5



with pyrrolidine gave 68% of the mono-substituted product **40**, but also afforded the amide **41** of **40** in 32% yield. Cleavage of the acetamido group during the substitution of **42** led to mixtures of products (Scheme 5).

Nucleophilic amination at a *meta*-substituted leaving group

The *meta*-amination yields were considerably increased under these modified reaction conditions for nucleophilic substitution and were superior to literature precedents[1] (Table 4), except those for indoles and indolines.[12] Consideration was thus given as to whether an alternative reaction mechanism was involved. The three main mechanisms used[1,2] to explain nucleophilic aromatic substitution reactions were not supported by the experimental evidence above. The S_N1 mechanism is inappropriate, and radical and photochemical mechanisms were rejected because no radical initiator was present, and reaction proceeded in the dark to give the same amination yields. The involvement of a benzyne intermediate was discounted because: a) the base used

Table 4 Comparison with literature[13] substitutions for the synthesis of **23**

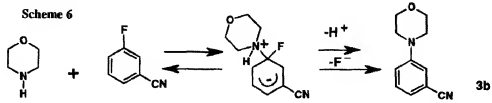
23

Reaction conditions	% Yield of 23
24 h reflux, MeCN, (ref. 12)	0
62 h, 10 kbar, MeCN, (ref. 12)	77
60 h, 100 °C, DMSO	98

(morpholine) was too weak; b) fluoride is too poor a leaving group; and c) there was no evidence by TLC, ^{1}H-NMR or ^{13}C-NMR of *cine*-substituted reaction products in any of these substitutions.

In conventional *ortho*/*para* nucleophilic substitutions the intermediate anion is stabilised[1] *via* both canonical resonance structures and the field effects of the EWG. With the *meta*-substituted substrates described

Scheme 6



3b

in this paper resonance is restricted, and mainly the field effect remains for the stabilisation of the postulated cyclohexadienyl anion reaction intermediate (Scheme 6). This hypothesis of field effect stabilisation is supported by: a) the yield variation (Table 3) being related to the Hammett σ-*meta* substituent values of the EWGs e.g. NO_2 >CN >CF_3 , which explains the restriction of the reaction to strong EWGs such as cyano and nitro or

combinations of EWGs with a similar capacity for stabilisation; b) the known[14, 15] substitution by the stronger (and anionic) oxygen nucleophiles (such as phenoxide ions) in the presence of strong EWGs. Thus, pending further kinetic investigation, the S_NAr mechanism was retained as an explanation for the high yielding *meta*-aminations reported herein, with the EWGs stabilising the intermediate cyclohexadienyl anion by field effects.

Conclusions

The enhanced yields of *meta*-substituted products in these nucleophilic amination reactions were based on the identification of favourable reaction conditions, because the precedented poor nucleophilic substitution yields were obtained in our preliminary work (Table 1). The high yielding, but slow substitution process was influenced by: the amine concentration; the nature of the substrate EWG; the leaving group (fluoro being preferred to triflate) and the choice of solvent. Best yields were obtained with cyclic secondary amines, but primary alkylamines containing a δ-substituted heteroatom also afforded adequate yields. The reported general procedure represents an alternative synthetic approach to the well established palladium catalyzed amination methods, but is restricted to benzenes substituted with an EWG. The yields obtained in this laboratory (with an

Table 5 A comparison of catalyzed and non-catalyzed substitutions

Pd-catalyzed			Uncatalyzed 60 h, DMSO		
EWG	**R**	**% Yield**	**EWG**	**R**	**% Yield**
2-CN	Br	**3a** 90	2-CN	F	**3a** 90
3-CN	Br	**3b** 72	3-CN	F	**3b** 62
4-CN	Br	**3c** 84	4-CN	F	**3c** 97

optimal leaving group, but without using specialist organometallic chemistry apparatus or optimising the palladium catalyst) were very similar for both sets of reaction conditions (Table 5). The nitro- and cyano-substituted products described above offer scope for further transformations to other C- and N-ring substituted aniline derivatives.

Experimental

Melting points were determined with a Buchi apparatus and are uncorrected. The ^{1}H-NMR spectra were determined with a Bruker AM (300 MHz) spectrometer and the ^{13}C-NMR with a Bruker DPX (400 MHz) instrument. Mass spectra were measured on a Platform Micromass (electrospray) instrument, and IR spectra as

Nujol mulls on a Perkin Elmer series 1600 FTIR instrument. Reactions were carried out under an atmosphere of argon and column chromatography was on E. Merck silica gel (Kieselgel 60, 230-400 mesh). All compounds analysed correctly for C, H, and N, (±0.4%), and the ^{1}H- and ^{13}C-NMR spectra were consistent with the assigned structures.

Example of the uncatalyzed substitution method used in Table 1

3-(4-Morpholino)benzonitrile (3b).

Morpholine (0.39 mL, 4.5 mmol) was added to a stirred suspension of K_2CO_3 (0.840 g, 6 mmol), 3-fluorobenzonitrile (0.363 g, 3 mmol) in DMSO (5 ml) at 20 °C. The reaction mixture was flushed with argon and heated to 105 °C for 18 h, cooled and water (50 mL) added. The aqueous was extracted with diethyl ether (3 x 40 mL). The organic layers were combined, washed with saturated brine (2 x 40 mL), dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography eluting with ethyl acetate-isohexane (1:9 v/v) to give **3b**, as a cream solid, (0.08 g, 14%), mp 102-103 °C; Rf 0.31 silica gel (ethyl acetate-isohexane 1:3 v/v); {Found: C, 70.2; H, 6.6; N, 14.9. $C_{11}H_{12}N_2O$ requires C, 70.0; H, 6.4; N, 15.0 %}; δ_H ($CDCl_3$) 7.12 (m, 1H), 7.02 (m, 3H), 3.78 (t, 4H, J = 4.8 Hz), 3.10 (t, 4H, J = 5.1 Hz); δ_C (400 MHz; $DMSOd_6$) 151.3 (s), 129.9 (s), 122.8 (s), 119.5 (s), 119.1 (s), 118.1 (s), 113.0 (s), 66.5 (s), 48.4 (s); IR (cm^{-1}) 2226, 1599, 1573, 1307, 1270, 1244, 1116, 994, 961; m/z (M+H) 189.

Ethyl 3-(4-morpholino)benzoate, palladium-catalyzed method (2b).

Ethyl 3-bromobenzoate(1.92 mL, 12 mmol) was added to a stirred, degassed, anhydrous solution of BINAP (0.336 g, 4.5 mol%), NaOBu^t (1.615 g, 16.8 mmol), tris(dibenzylideneacetone)dipalladium(0), $Pd_2(dba)_3$ (0.33 g, 3 mol%) and morpholine (1.25 mL, 14.4 mmol), in toluene (30 mL). The reaction mixture was stirred at 90 °C for 20 h under argon. The solution was cooled, and extracted with 1 M HCl (2 x 40 mL), the aqueous phase was basified with aqueous Na_2CO_3 and extracted with ethyl acetate (3 x 50 mL). The organic phase was washed with saturated brine (1 x 50 mL), dried and concentrated to give a brown oil. The oil was purified by chromatography on silica gel. Elution with methanol-dichloromethane (1:50 v/v) gave **4** as a brown oil, (0.10 g, 7 %); δ_H ($CDCl_3$) 7.39 (m, 5H), 3.65 (broad t, 4H), 3.53 (broad t, 4H); m/z (M+H) 192. Further elution with methanol-dichloromethane (1:25 v/v) gave, as a yellow oil **2b**; (0.15 g, 6 %); δ_H ($CDCl_3$) 7.51 (s, 1H), 7.48(d, 1H, J = 2.8 Hz), 7.25 (t, 1H, J = 4.8 Hz), 7.0 (dd, 1H, J = 7.8 and 1.9 Hz), 4.3 (q, 2H, J = 7.1 Hz), 3.8 (t, 4H, J = 5.1 Hz), 3.13 (t, 4H, J = 4.7 Hz), 1.33 (t, 3H, J = 7.1 Hz); m/z (M+H) 236. Further elution with methanol-dichloromethane (1:12 v/v) gave **5**, as a dark brown oil (0.45 g, 28%); δ_H ($CDCl_3$) 7.30 (m, 1H), 6.97 (m, 2H), 6.83 (d, 1H, J = 5.9 Hz), 3.85 (t, 4H, J = 4.7 Hz), 3.68 (m, 8H), 3.20 (t, 4H, J = 5.2 Hz); m/z (M+H) 277.

3-(4-Morpholino)benzonitrile, palladium-catalyzed method (3b).

3-Bromobenzonitrile (10 g, 55 mmol) was added to a stirred, degassed, solution of BINAP (1.54 g, 4.5 mol%), NaOBu^t (7.4 g, 77 mmol), $Pd_2(dba)_3$ (1.51 g, 3 mol%) and morpholine (5.74 mL, 66 mmoles), in anhydrous toluene (150 mL). The reaction mixture was stirred at 90 °C for 20 h under argon. On cooling the mixture was extracted with 1 M HCl (3 x 50 mL), the aqueous phase was basified with aqueous Na_2CO_3 and extracted with ethyl acetate. The combined extracts were washed with saturated brine (1 x 50 mL), dried and concentrated to give a brown solid. The original toluene organic phase was exaporated and the residue combined with the brown solid and was purified by flash column chromatography. The column was eluted with ethyl acetate-isohexane (1:5 v/v) giving **3b** as a cream solid, (7.48 g, 72%); mp 102-103 °C; δ_H ($CDCl_3$) 7.29 (dd, 1H, J = 7.1 and 8.9Hz), 7.08 (s, 1H), 7.03 (m, 2H), 3.81 (t, 4H, J = 4.9 Hz), 3.11 (t, 4H, J = 5.0 Hz); m/z (M+H) 189.

t-Butyl 3-(4-morpholino)benzoate (7).

t-Butyl 3-bromobenzoate (0.77 g, 3 mmol) was added to a stirred, degassed, solution of BINAP (0.084 g, 4.5 mol%), NaOBu^t (0.4 g, 4.2 mmol), $Pd_2(dba)_3$ (0.082 g, 3 mol%) and morpholine (0.30 g, 3.3 mmol), in anhydrous toluene (30 mL). The reaction mixture was stirred at 90 °C for 20 h under argon. On cooling the mixture was filtered and the solvent evaporated to give a dark brown residue. The residue was triturated in ethyl acetate-isohexane (1:9 v/v) and refiltered. The filtrate was evaporated to give an oil which was purified by column chromatography on a IST Bond Elut (25 g silica gel). The column was eluted with ethyl acetate-isohexane (1:9 v/v) to give **7** as an oil, (0.556 g, 71%); δ_H ($CDCl_3$) 7.48 (t, 1H, J = 1.9 Hz), 7.42 (d, 1H, J = 5.3 Hz), 7.23 (t, 1H, J = 7.5 Hz), 6.98 (dd, 1H, J = 9.0 and 2.5 Hz), 3.80 (t, 4H, J = 4.8 Hz), 3.11 (t, 4H, J = 4.9 Hz) 1.52 (s, 9H); m/z (M+H) 264.

3-(4-Morpholino)benzoic acid (1b).

a) Trifluoroacetic acid (5 mL, 64.9 mmol), was added dropwise to a stirred solution of t-butyl-3-(4-morpholino)benzoate (0.50 g, 1.89 mmol) in dichloromethane (10 mL), at 0 °C under argon. The mixture was stirred for 1 h, evaporated to an oil, and toluene (30 mL) was added. Evaporation gave an oil which solidified to give **1b** (0.43 g, 68.5 %) as the trifluoroacetate salt; m/z (M-H) 206.

b) 5M NaOH (33.87 mL, 169.4 mmol) was added to a stirred suspension of **3b** (6.37 g, 33,7 mmol) in butan-1-ol (30 mL) and the mixture stirred at 115 °C under argon for 9 h. On cooling the butanol was evaporated and the reaction mixture neutralised with an equivalent amount of 1M HCl (169 mL, 169 mmol). The resulting colourless precipitate was filtered, washed with water, diethyl ether and dried to give **1b** as a colourless solid (6.443 g, 92%) 164-165 °C; [Found : C, 63.4; H, 6.3; N, 6.6 $C_{11}H_{13}NO_3$ requires C, 63.7; H, 6.2; N, 6.8%]; δ_H (DMSO-d_6) 12.8 (s, 1H), 7.44 (s, 1H), 7.32 (t, 1H, J = 8.0 Hz), 7.20 (d, 2H, J = 7.3 Hz), 3.77 (t, 4H, J = 4.8 Hz), 3.13 (t, 4H, J = 5.0 Hz); m/z (M-H) 206.

Example of the General Procedure for uncatalyzed substitution in DMSO at 100 °C for 60h.

3-(4-Morpholino)benzonitrile (3b).

Morpholine (72 mL, 0.742 mol), was added to a solution of 3-fluorobenzonitrile (16.34 g, 0.135 mol) in DMSO (112 mL) and the mixture heated for 60 h at 100 °C. The reaction mixture was cooled and poured into water (1200 mL). The solid product was filtered, washed with water and dried *in vacuo* giving a pale pink solid (14.69 g). The residual aqueous filtrate was extracted with diethyl ether (2 x 400 mL). The combined organic phases were washed with saturated brine, dried ($MgSO_4$) and the extracts evaporated giving an oil (6.7 g). The oil was purified by filtration chromatography on silica gel. Elution with ethyl acetate-isohexane (1:9 v/v) gave **3b** as a colourless solid (1.02 g). (15.71 g 62%); mp 102-103 °C; [Found: C, 70.0; H, 6.4; N, 15.0. $C_{11}H_{12}N_2O$ requires C, 70.2; H, 6.6; N, 14.9 %]; δ_H ($CDCl_3$) 7.29 (dd, 1H, J = 9.0 and 7.2 Hz), 7.08 (s, 1H), 7.03 (m, 2H), 3.81 (t, 4H, J = 4.5 Hz), 3.11 (t, 4H, J = 4.8 Hz); m/z (M+H) 189.

This General Procedure was used to synthesize the compounds in Table 6 below.

Table 6 Reactions of morpholine under the General Procedure

Cmpd	mp °C	yield %	m/z M+H	cmpd	mp °C	yield %	m/z M+H
3a	59-60	90	189	**24**	105-106	74	304
3c	81-82[a]	97	189	**25**	165-166	97	257
13	109-110	14	243	**26**	oil	19	232
14	221-223	36	253 (M+)	**27**	oil	3	236
15	oil	68	187	**30**	103-104[c]	8	182
16	oil	69	202	**31**	75-77	1	223
17	84-85	64	173	**33**	75-77	20	223
18a	oil	22	223	**34**	72-73	4	203
19a	oil	10	174	**35**	103-104	78	207
19b	oil	58	217	**36**	195-196	16	274
19c	oil	34	190	**37**	135-136	62	225
19d	oil	36	204	**38**	205-206	37	294
19e	oil	39	217 (M+)	**39**	67-68	77	254
20a	oil	42	191	**40**	91-93	68	263
20b	oil	38	231	**41**	53-55	32	238
20c	oil	31	206 (M+)	**43**	181-183[d]	29	266
21	236-237	68	186	**44**	164-165	3	224
22	234-235	17	288	**45**	91-93	16	157
23	103-105[b]	98	181				

[a] i Reference 12a mp 75-76.5 °C; ii Wolfe, J. P.; Buchwald, S. L. *J.Org.Chem.* **1997**, *62*, 1264-1267, mp 54-55 °C. [b] Hurst, D. T. *Heterocycles*, **1988**, *27*, 371-376, mp 106.5 °C. [c] Popp, F.D.; et al. *J. Med. Chem.* **1967**, *10*, 481-484 , mp 100-105 °C. [d] Ainsworth, D. P.; Suschitzky, H. *J Chem Soc. C.* 1966, 111-113. mp 160 °C.

2-(4-Morpholino)benzonitrile (3a).

In a similar manner 2-fluorobenzonitrile was reacted with morpholine, to give **3a**, as a cream solid, (0.462 g, 82 %); which was crystallised from isohexane; mp 59-60 °C; R_f 0.39 silica gel (ethyl acetate-isohexane 1:3 v/v);

Table 7 Microanalyses for compounds in Table 6

	Found %				Reqd %		
Compd	**C**	**H**	**N**	**Formulae**	**C**	**H**	**N**
3a	69.9	6.4	14.8	$C_{11}H_{12}N_2O$	70.0	6.4	15.0
3c	70.0	6.7	14.9	$C_{11}H_{12}N_2O$	70.0	6.4	15.0
13	49.7	4.7	11.3	$C_{10}H_{11}N_2O_2Cl$	49.6	4.5	11.6
14	47.1	4.1	16.3	$C_{10}H_{11}N_3O_4$	47.2	4.3	16.1
15	76.5	7.4	14.9	$C_{12}H_{14}N_2.0.1H_2O$	76.5	7.5	14.8
16	69.9	7.0	20.3	$C_{12}H_{13}N_5.0.25H_2O$	70.0	7.4	20.4
17	76.4	7.2	16.2	$C_{11}H_{12}N_2$	76.7	7.0	16.3
18a	80.7	6.4	12.5	$C_{13}H_{14}N_2$	81.1	6.3	12.6
19a	76.2	8.1	16.0	$C_{11}H_{16}N_2$	75.8	8.0	16.3
19b	72.0	9.2	19.7	$C_{13}H_{19}N_3$	71.3	8.8	19.4
19c	69.6	8.1	22.1	$C_{11}H_{17}N_3$	69.8	7.9	22.2
19d	68.9	8.7	19.8	$C_{12}H_{17}N_5.0.25H_2O$	69.3	8.7	20.2
19e	60.4	8.9	19.3	$C_{13}H_{19}N_3$	60.8	8.8	19.4
20a	69.2	7.5	14.4	$C_{11}H_{14}N_2O$	69.5	7.4	14.7
20b	72.3	8.8	12.0	$C_{16}H_{26}N_2O$	72.4	8.6	12.1
20c	64.0	6.9	13.4	$C_{11}H_{14}N_2S$	64.1	6.8	13.6
21	50.9	6.0	16.5	$C_{13}H_{17}N_3$	50.8	5.8	16.2
22	72.1	5.6	19.0	$C_{18}H_{16}N_4.0.7H_2O$	71.8	5.8	18.6
23	57.5	5.7	13.3	$C_{16}H_{12}N_2O_3$	57.6	5.8	13.5
24	55.4	5.3	4.4	$C_{16}H_{18}FNO_3$	55.4	5.3	4.6
25	55.9	4.4	10.8	$C_{12}H_{11}F_3N_2O$	56.3	4.3	10.9
26	56.4	5.0	5.8	$C_{11}H_{12}F_3NO$	56.7	5.2	6.0
27	66.0	7.0	6.1	$C_{12}H_{17}NO_3$	66.4	7.2	6.0

Table 7 contd

30	66.6	6.5	7.5	$C_{10}H_{11}FNO$	66.3	6.6	7.7
31	59.3	6.4	12.5	$C_{11}H_{14}N_2O_3$	59.5	6.3	12.6
33	59.1	6.3	12.4	$C_{11}H_{14}N_2O_3$	59.5	6.3	12.6
34	69.8	7.1	13.5	$C_{12}H_{14}N_2O.0.1H_2O$	69.7	7.0	13.6
35	64.1	5.4	13.5	$C_{11}H_{11}FN_2O$	64.0	5.4	13.6
36	65.9	7.0	15.4	$C_{13}H_{19}N_3O_2$	65.8	6.8	15.2
37	53.2	4.7	12.1	$C_{10}H_{11}FN_2O_3$	53.1	4.9	12.3
38	52.0	6.2	14.2	$C_{14}H_{19}N_3O_4$	52.3	6.5	14.3
39	61.8	6.4	5.5	$C_{19}H_{19}FNO_3$	61.7	6.3	5.5
40	65.6	6.9	6.0	$C_{19}H_{18}FNO_2$	65.8	6.8	6.0
41	68.3	7.3	10.4	$C_{19}H_{19}FNO_2$	68.7	7.3	10.7
43	54.6	5.7	15.6	$C_{12}H_{15}N_3O_4$	54.3	5.7	15.8
44	53.6	5.6	18.5	$C_{10}H_{13}N_3O_4$	53.8	5.8	18.8
45	46.0	3.4	17.8	$C_6H_3FN_2O_2$	46.1	3.2	17.9

[Found: C, 69.9; H, 6.4; N, 14.8. $C_{11}H_{12}N_2O$ requires C, 70.0; H, 6.4; N, 15.0 %]; δ_H ($CDCl_3$) 7.53 (dd, 1H, *J* = 8.0 and 1.9 Hz), 7.42 (td, 1H, *J* = 7.6 and 1.4 Hz), 6.95 (m, 2H), 3.83 (t, 4H, *J* = 4.8 Hz), 3.16 (t, 4H, *J* = 4.7 Hz); IR (cm^{-1}) 2217, 1594, 1484, 1283, 1255, 1224, 1112, 1937, 936, 922, 758; m/z (M+H) 189.

4-(4-Morpholino)benzonitrile (3c).

In a similar manner 4-fluorobenzonitrile was reacted with morpholine, to give **3c**, as a cream solid, (0.523 g, 94 %); which was crystallised from ethyl acetate-isohexane (1:10 v/v); mp 82-83 °C; R_f 0.23 silica gel (ethyl acetate-isohexane 1:3 v/v); [Found: C, 70.0; H, 6.7; N, 14.9. $C_{11}H_{12}N_2O$ requires C, 70.0; H, 6.4; N, 15.0%]; δ_H ($CDCl_3$) 7.42 (d, 2H, *J* = 8.9 Hz), 6.80 (d, 2H, *J* = 9.0 Hz), 3.78 (t, 4H, *J* = 5.0 Hz), 3.21 (t, 4H, *J* = 4.8 Hz); IR (cm^{-1}) 2216, 1605, 1515, 1244, 1181, 1115, 928; m/z (M+H) 189.

***N*-4-(3-chloro-5-nitro)morpholine and *N*-4-(3,5-dinitrophenyl)morpholine (13 and 14).**

Morpholine (0.718 mL, 8.25 mmol) was added to a stirred solution of 3,5-dinitro chlorobenzene (0.292 g, 1.43 mmol) in DMSO (2.5 mL), causing a colour change from cream to purple. The mixture was heated at 100 °C and stirred for 60 h under an argon atmosphere. The cooled reaction mixture was poured into water (25 mL), and the aqueous phase extracted with ethyl acetate. The combined extracts were washed with saturated brine, dried ($MgSO_4$) and evaporated *in vacuo* giving an orange solid. The residue was purified on a 10g silica Bond Elut, eluting with ethyl acetate-isohexane (1:9 v/v), gave **13**, as an orange solid, (0.047 g, 14 %); mp 109 - 110°C; [Found: C, 49.7; H, 4.7; N, 11.3. $C_{10}H_{11}FN_2O_2Cl$ requires C, 49.6; H, 4.5; N, 11.6%]; δ_H ($CDCl_3$) 7.58 (d, 1H, *J* = 1.9 Hz), 7.50 (d, 1H, *J* = 2.0 Hz), 7.02 (d, 1H, *J* = 1.9 Hz), 3.78 (t, 4H, *J* = 1.8 and 3.3 Hz), 3.18

(t, 4H, J = 1.8 and 3.3 Hz); m/z (M+H) 243. Further elution with ethyl acetate-isohexane (1:3 v/v) gave, as an orange solid, **14** (0.127 g, 35 %); mp 221-223°C; [Found: C, 47.1; H, 4.1; N, 16.3. $C_{10}H_{11}N_3O_4$ requires C, 47.2; H, 4.3; N, 16.1%]; δ_H ($CDCl_3$) 8.35 (s, 1H), 7.88 (s, 2H), 3.82 (t, 4H, J = 4.8 Hz), 3.30 (t, 4H, J = 4.6 Hz); m/z (M^+) 253.

3-Fluoro-5-morpholinobenzonitrile (35) and 3,5-dimorpholinobenzonitrile (36).

In a similar manner and on the same scale as **37** below, eluting with ethyl acetate-isohexane (1:4 v/v), gave **35**, as a colourless solid, (0.484 g, 78 %); mp 112.5 -113.5 °C; [Found: C, 64.1; H, 5.4; N, 13.5. $C_{11}H_{11}FN_2O$ requires C, 64.0; H, 5.4; N, 13.6 %]; δ_H ($CDCl_3$) 7.54 (s, 1H), 7.29 (d, 1H, J = 2.0 Hz), 6.82 (d, 1H, J = 2.1 Hz), 3.88 (t, 4H, J = 5.1 Hz), 3.23 (t, 4H, J = 4.8 Hz); m/z (M+H) 207.4. Further elution with ethyl acetate-isohexane (1:1 v/v) gave, as a colourless solid, **36** (0.13 g, 16 %); mp 196-198 °C; [Found: C, 65.9; H, 7.0; N, 15.4. $C_{15}H_{19}N_3O_2$ requires C, 65.8; H, 6.8; N, 15.2%]; δ_H ($CDCl_3$) 6.65 (d, 2H, J = 1.8 Hz), 6.58 (t, 1H, J = 2.0 and 1.9 Hz), 3.83 (t, 8H, J = 4.9 Hz), 3.17 (t, 8H, J = 5.1 Hz); m/z (M+H) 274.5.

***N*-4-(3-fluoro-5-nitrophenyl)morpholine (37) and 3,5-dimorpholinonitrobenzene (38).**

Morpholine (1.437 mL, 6.5 mmol), was added to a solution of 3,5-difluoronitrobenzene (0.477 g, 3 mmol) in DMSO (5 mL) and the mixture heated for 60 h at 100 °C. After cooling, the reaction mixture was poured into water (50 mL) and an orange solid product collected. The residual aqueous filtrate was extracted with diethyl ether (3x50 mL) and the combined extracts were washed with saturated brine, dried ($MgSO_4$) and evaporated giving orange solid. The solids were combined and purified by silica filtration chromatography. Elution with ethyl acetate-isohexane (1:4 v/v) gave, as an orange solid, **37** (0.42 g; 62 %); mp 134-135 °C; [Found: C, 53.2; H, 4.7; N, 12.1. $C_{10}H_{11}FN_2O_3$ requires C, 53.1; H, 4.9; N, 12.3 %]; δ_H ($CDCl_3$) 7.54 (s, 1H), 7.29 (d, 1H, J = 5.1 Hz), 6.82 (d, 1H, J = 5.8 Hz), 3.88 (t, 4H, J = 4.9 Hz), 3.23 (t, 4H, J = 5.0 Hz); m/z (M+H) 227. Further elution with ethyl acetate-isohexane (1:1 v/v) gave, as an orange solid, **38** (0.325 g, 37 %); mp 207-209 °C; [Found: C, 52.0; H, 6.2; N, 14.2. $C_{14}H_{18}N_3O_4$ requires C, 52.3; H, 6.5; N, 14.3 %]; δ_H ($CDCl_3$) 7.26 (s, 2H), 6.63 (s, 1H), 3.90 (t, 8H, J = 4.8 Hz), 3.21 (t, 8H, J = 5.0 Hz); m/z (M+H) 294.

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Exhibit N

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=> s 2359-60-6/rn
L1            1 2359-60-6/RN

=> d

L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2007 ACS on STN
RN   2359-60-6  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   Benzenamine, 4-(1-piperidinyl)-  (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   Piperidine, 1-(p-aminophenyl)- (6CI, 7CI, 8CI)
OTHER NAMES:
CN   1-(4-Aminophenyl)piperidine
CN   1-(p-Aminophenyl)piperidine
CN   4-(1-Piperidino)aniline
CN   4-(1-Piperidinyl)aniline
CN   4-(1-Piperidyl)aniline
CN   4-(Piperidin-1-yl)phenylamine
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LC   STN Files:   BEILSTEIN*, BIOSIS, CA, CAOLD, CAPLUS, CASREACT, CHEMCATS,
       CSCHEM, IFICDB, IFIPAT, IFIUDB, TOXCENTER, USPAT2, USPATFULL
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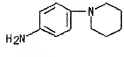

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             134 REFERENCES IN FILE CAPLUS (1907 TO DATE)
               4 REFERENCES IN FILE CAOLD (PRIOR TO 1967)


=> s 2524-67-6/rn
L2            1 2524-67-6/RN

=> d

L2   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2007 ACS on STN
RN   2524-67-6  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   Benzenamine, 4-(4-morpholinyl)-  (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   Morpholine, 4-(p-aminophenyl)- (6CI, 7CI, 8CI)
OTHER NAMES:
CN   (4-(Morpholino)phenyl)amine
CN   4-(4-Aminophenyl)morpholine
CN   4-(4-Morpholinyl)aniline
CN   4-(4-Morpholinyl)benzenamine
CN   4-(Morpholin-4-yl)phenylamine
CN   4-Morpholinoaniline
CN   4-Morpholinobenzenamine
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CN   4-N-Morpholinoaniline
CN   N-(4'-Aminophenyl)morpholine
CN   N-(4-Aminophenyl)morpholine
CN   NSC 26334
CN   p-Morpholinoaniline
DR   131852-32-9
MF   C10 H14 N2 O
CI   COM
LC   STN Files:   BEILSTEIN*, BIOSIS, CA, CAOLD, CAPLUS, CASREACT, CHEMCATS,
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       TOXCENTER, USPAT2, USPATFULL, USPATOLD
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     Other Sources:   EINECS**, NDSL**, TSCA**
         (**Enter CHEMLIST File for up-to-date regulatory information)
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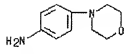

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=> s 55048-24-3/rn
L3              1 55048-24-3/RN

=> d

L3   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2007 ACS on STN
RN   55048-24-3  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   Benzenamine, 3-chloro-4-(4-morpholinyl)-  (CA INDEX NAME)
OTHER NAMES:
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CN   3-Chloro-4-(4-morpholinyl)aniline
CN   3-Chloro-4-morpholinoaniline
CN   4-(4-Amino-2-chlorophenyl)morpholine
CN   N-(4-Amino-2-chlorophenyl)morpholine
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LC   STN Files:   BEILSTEIN*, CA, CAPLUS, CASREACT, CHEMCATS, CHEMLIST, CSCHEM,
       IFICDB, IFIPAT, IFIUDB, TOXCENTER, USPAT2, USPATFULL
         (*File contains numerically searchable property data)
     Other Sources:   EINECS**, NDSL**, TSCA**
         (**Enter CHEMLIST File for up-to-date regulatory information)
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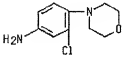

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              34 REFERENCES IN FILE CAPLUS (1907 TO DATE)


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L4            1 1204-85-9/RN

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RN   1204-85-9  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   Benzonitrile, 4-(1-piperidinyl)-  (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   Benzonitrile, p-piperidino- (7CI, 8CI)
OTHER NAMES:
CN   1-(p-Cyanophenyl)piperidine
CN   4-(1-Piperidinyl)benzonitrile
CN   4-Piperidinobenzonitrile
CN   N-(4-Cyanophenyl)piperidine
CN   p-Piperidinobenzonitrile
MF   C12 H14 N2
LC   STN Files:   BEILSTEIN*, CA, CAOLD, CAPLUS, CASREACT, CHEMCATS,
       CHEMINFORMRX, TOXCENTER, USPAT2, USPATFULL
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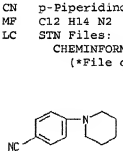


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             79 REFERENCES IN FILE CAPLUS (1907 TO DATE)
              3 REFERENCES IN FILE CAOLD (PRIOR TO 1967)


=> s 10282-31-2
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                (10282-31-2/RN)

=> d

L5   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2007 ACS on STN
RN   10282-31-2  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   Benzonitrile, 4-(4-morpholinyl)-  (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   Benzonitrile, p-morpholino- (7CI, 8CI)
OTHER NAMES:
CN   4-(4-Cyanophenyl)morpholine
CN   4-(Morpholin-4-yl)benzonitrile
CN   4-Morpholinobenzonitrile
CN   N-(4-Cyanophenyl)morpholine
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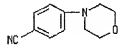
CN NSC 698610
MF C11 H12 N2 O
LC STN Files: BEILSTEIN*, CA, CAOLD, CAPLUS, CASREACT, CHEMCATS, CHEMINFORMRX, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

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81 REFERENCES IN FILE CAPLUS (1907 TO DATE)
1 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

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Exhibit O

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=> s 2359-60-6/rn
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=> file caplus
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.45       1.08

FILE 'CAPLUS' ENTERED AT 16:36:00 ON 28 AUG 2007
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2007 AMERICAN CHEMICAL SOCIETY (ACS)
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FILE COVERS 1907 - 28 Aug 2007  VOL 147 ISS 10
FILE LAST UPDATED: 27 Aug 2007  (20070827/ED)

Effective October 17, 2005, revised CAS Information Use Policies apply.
They are available for your review at:

http://www.cas.org/infopolicy.html

=> s L1
L2          134 L1

=> d 134

L2   ANSWER 134 OF 134  CAPLUS  COPYRIGHT 2007 ACS on STN
     Full Text   Citing References
AN   1948:38890  CAPLUS
DN   42:38890
OREF 42:8255e-i
TI   The effect of Tween 80 in vitro on the bacteriostatic activity of twenty
     compounds for Mycobacterium tuberculosis
AU   Youmans, Anne S.; Youmans, Guy P.
CS   Northwestern Univ. Med. School, Chicago
SO   Journal of Bacteriology (1948), 56, 245-52
     CODEN: JOBAAY; ISSN: 0021-9193                                  1948
DT   Journal
LA   Unavailable

=> s 2524-67-6/rn
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USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.

FILE COVERS 1907 - 28 Aug 2007 VOL 147 ISS 10
FILE LAST UPDATED: 27 Aug 2007 (20070827/ED)

Effective October 17, 2003, revised CAS Information Use Policies apply.
They are available for your review at:

http://www.cas.org/infopolicy.html

=> d 431

L3 ANSWER 431 OF 431 CAPLUS COPYRIGHT 2007 ACS on STN
Full Text | Citing References

AN 1935:39415 CAPLUS
DN 29:39415
OREF 29:5129d-e
TI N-(p-Aminophenyl)morpholine
IN Lubs, Herbert A.
PA E. I. du Pont de Nemours & Co.
DT Patent
LA Unavailable
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2004763		19350611	US 1932-616745	19320611

1932

=> s 55048-24-3/rn
34 55048-24-3
0 55048-24-3D
L4 34 55048-24-3/RN
(55048-24-3 (NOTL) 55048-24-3D)

=> d 34

L4 ANSWER 34 OF 34 CAPLUS COPYRIGHT 2007 ACS on STN
Full Text | Citing References

AN 1975:170926 CAPLUS
DN 82:170926

TI 9-Aminoimidazo[4,5-f]quinoline derivatives
IN Spencer, Claude F.; Snyder, Harry R., Jr.
PA Morton-Norwich Products, Inc.
SO Ger. Offen., 20 pp.
CODEN: GWXXBX
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 2427410	A1	19750109	DE 1974-2427410	19740605
	US 3878206	A	19750415	US 1973-367498	19730606
	ZA 7400798	A	19750924	ZA 1974-798	19740206
	AU 7465452	A	19750814	AU 1974-65452	19740211
	GB 1402243	A	19750806	GB 1974-8717	19740226
	SE 7405653	A	19741209	SE 1974-5653	19740426
	SE 391926	B	19770307		
	CH 598257	A5	19780428	CH 1974-6650	19740515
	NL 7406572	A	19741210	NL 1974-6572	19740516
	JP 50019775	A	19750301	JP 1974-58879	19740527
	JP 57038593	B	19820816		
	FR 2232318	A1	19750103	FR 1974-19424	19740605
	BE 816004	A1	19741206	BE 1974-145153	19740606
	DK 7403020	A	19750203	DK 1974-3020	19740606
	DK 133104	B	19760322		
PRAI	US 1973-367498	A	19730606		

1973

=> s 1204-85-9/rn
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1 1204-85-9D
L5 78 1204-85-9/RN
(1204-85-9 (NOTL) 1204-85-9D)

=> d 78

L5 ANSWER 78 OF 78 CAPLUS COPYRIGHT 2007 ACS on STN
Full Text | Citing References
AN 1965:36301 CAPLUS
DN 62:36301
OREF 62:6360e-g
TI Effect of the leaving group on the velocity of nucleophilic aromatic substitutions
AU Suhr, Harald
CS Univ. Tuebingen, Germany
SO Chemische Berichte (1964), 97(12), 3268-76
CODEN: CHBEAM; ISSN: 0009-2940
DT Journal
LA German

1964

=> s 10282-31-2/rn
81 10282-31-2
0 10282-31-2D
L6 81 10282-31-2/RN
(10282-31-2 (NOTL) 10282-31-2D)

=> d 81

L6 ANSWER 81 OF 81 CAPLUS COPYRIGHT 2007 ACS on STN

Full Text | Citing References

AN 1966:437896 CAPLUS
DN 65:37896
OREF 65:7008g-h,7009a
TI Nucleophilic aromatic substitutions with amines
AU Suhr, Harald; Grube, Helmut
CS Univ. Tuebingen, Germany
SO Berichte der Bunsen-Gesellschaft (1966), 70(5), 544-50
CODEN: BBPCAX; ISSN: 0940-483X
DT Journal
LA German

1966

=>

Exhibit P

UNITED STATES PATENT OFFICE

2,004,763

N (p-AMINO-PHENYL) MORPHOLINE AND METHOD OF PREPARING THE SAME

Herbert A. Lubs, Wilmington, Del., assignor to E. I. du Pont de Nemours & Company, Wilmington, Del., a corporation of Delaware

No Drawing. Application June 11, 1932, Serial No. 616,745

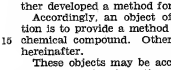
6 Claims. (Cl. 260—28)

This invention relates to a new organic compound and a method of preparing the same.

Aromatic amino compounds containing a substituted amino group in the para position are well known intermediates in the production of dyes. The aromatic amino body containing a morpholine nucleus in the para position to the amino group has not been known until the present time. We have discovered such a compound and found that it is very valuable, particularly as an intermediate in the production of dyes and have further developed a method for its manufacture.

Accordingly, an object of the present invention is to provide a method for preparing a new chemical compound. Other objects will appear hereinafter.

These objects may be accomplished according to the following invention which comprises reducing N(p-nitroso-phenyl) morpholine or N(p-nitro-phenyl) morpholine to produce N(p-amino-phenyl) morpholine which is the new chemical compound. The N(p-nitroso-phenyl) morpholine has been described in German Patent 119,785. I have found that under suitable conditions this compound can be reduced to form a new compound N(p-amino-phenyl) morpholine having the probable formula:



Although various reducing agents may be employed under various conditions for reducing the N(p-nitroso-phenyl) morpholine, I have found that such reduction may be satisfactorily and economically effected by adding, to an acid solution of N(p-nitroso-phenyl) morpholine hydrochloride, a metal, such as zinc dust, which will react with the acid to liberate free hydrogen.

In order to more fully illustrate my invention and the preferred mode in which I contemplate carrying the same into effect, the following example is given:

Example.—36 grams of N(p-nitroso-phenyl) morpholine hydrochloride were added to 325 cc. of concentrated hydrochloric acid diluted with 2000 cc. of water. The solution was agitated and 71 grams of zinc dust were slowly added thereto during agitation, meanwhile maintaining the temperature below 30° C. When all of the zinc dust had been added and the reduction was complete the solution was then made slightly alkaline with sodium hydroxide. The alkaline solution was then heated to boiling to precipitate the zinc hydroxide which was then removed by filtration. If desired, the amino-morpholine derivative may be isolated by concentrating the hot solution in vacuo. For most purposes, however, the solution can be used directly after removal of the zinc sludge.

The above method may be modified in various ways, for example, other non-oxidizing acids may be substituted for the hydrochloric acid; other metals, such as iron, may be substituted for the zinc; and other alkaline materials may be substituted for the sodium hydroxide.

The reduction of N(p-nitro-phenyl) morpholine may be accomplished by the same method under substantially the same conditions as the N(p-nitroso-phenyl) morpholine.

Pure N(p-amino-phenyl) morpholine is a colorless crystalline solid melting at about 128.8—130.2° C. Its acetyl derivative is a white crystalline solid having a melting point of 211.2—212.4° C.

While I have disclosed a specific method of producing my new compound, employing certain reducing agents, under particular conditions, it is to be understood that various other methods of reduction may be employed and that the particular method described may be modified in many ways without departing from the spirit of my invention. Accordingly, the scope of my invention is to be limited solely by the appended claims construed as broadly as is permissible in view of the prior art.

I claim:

1. The process of producing N(para-amino-phenyl) morpholine which comprises treating a solution of a member of the group consisting of N(p-nitroso-phenyl) morpholine and N(p-nitro-phenyl) morpholine in a non-oxidizing acid with a metal such as will react with the acid to liberate free hydrogen.

2. The process of producing N(para-amino-phenyl) morpholine which comprises treating a solution of N(p-nitroso-phenyl) morpholine in a non-oxidizing acid with a metal such as will react with the acid to liberate free hydrogen.

3. The process of producing N(para-amino-phenyl) morpholine which comprises treating a hydrochloric acid solution of N(p-nitroso-phenyl) morpholine hydrochloride with a metal such as will react with the acid to liberate free hydrogen.

4. The process of producing N(para-amino-phenyl) morpholine which comprises slowly adding zinc dust to a hydrochloric acid solution of N(p-nitroso-phenyl) morpholine hydrochloride with agitation.

5. The process of producing N(para-amino-phenyl) morpholine which comprises slowly adding iron to a hydrochloric acid solution of N(p-nitroso-phenyl) morpholine hydrochloride with agitation.

6. N(para-amino-phenyl) morpholine.

HERBERT A. LUBS.

Exhibit Q

United States Patent [19]

Spencer et al.

[11] **3,878,206**

[45] **Apr. 15, 1975**

[54] **9-(SUBSTITUTED AMINO)IMIDAZO[4,5-f]QUINOLINES**

[75] Inventors: **Claude F. Spencer; Harry R. Snyder, Jr.,** both of Norwich, N.Y.

[73] Assignee: **Morton-Norwich Products, Inc.,** Norwich, N.Y.

[22] Filed: **June 6, 1973**

[21] Appl. No.: **367,498**

[52] **U.S. Cl.** ..... **260/247.5 EP;** 260/288 R

[51] **Int. Cl.** **C07d 33/52**

[58] **Field of Search** ..... 260/247.5 EP, 288 R

[56] **References Cited**

OTHER PUBLICATIONS

Ishiwata et al., Chem. Pharm. Bull., 17, (12), 2455–2460 (1969).

Primary Examiner—Lorraine A. Weinberger
Assistant Examiner—Richard D. Kelly
Attorney, Agent, or Firm—Anthony J. Franze

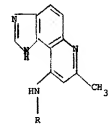
[57] **ABSTRACT**

A series of 9-(substituted amino)imidazo[4,5-f]quinolines are antibacterial agents, particularly effective against Haemophilus vaginalis a cause of bacterial vaginitis, and are adapted to be combined in pharmaceutical compositions.

22 Claims, No Drawings

9-(SUBSTITUTED AMINO)IMIDAZO[4,5-f]QUINOLINES

This invention is concerned with a series of 9-(substituted amino) imidazo[4,5-f]quinolines of the formula:



wherein R is 4-ethylphenyl, 4-(2-hydroxyethyl)phenyl, 4-n-dodecylphenyl, 9-anthryl, 4-chlorophenyl, 4-fluorophcnyl, 3-chlorophenyl, 4-acetylaminophenyl, 4-(N-methylacetylamino)phenyl, 4-(methylamino)phenyl, 4-aminophenyl, 4-morpholinophenyl, 4-pyrrolidinophenyl, 4-[N-methyl-N-(2-hydroxyethyl)amino]phenyl, 3-chloro-4-morpholinophenyl, 4-hydroxypcnyl, 3-hydroxyphenyl, 3-chloro-4-hydroxyphenyl, 4-carbethoxyphenyl, 4-benzoylphenyl, or 2-methoxy-5-acetylphenyl.

The compounds of this invention are prepared in accordance with methods described in the following examples. They are usually obtained in the form of hydrochloride salts.

EXAMPLE I

9-(p-Ethylanilino)-7-methyl-1H-imidazo[4,5-f]quinoline Hydrochloride

A. Ethyl 3-(5-Benzimidazolylamino)crotonate

An 82-g (0.5 mole) sampel of 5-nitrobenzimidazole in 900 ml of ethanol was reduced over 4 g of 5 percent Pd/C catalyst containing 50 percent H_2O. After filtration of the catalyst, 65 g (0.5 mole) of ethyl acetoacetate, 20 g of anhydrous calcium sulfate, and 0.5 ml of HOAc was added. After filtration, the solution was concentrated in vacuo till a solid remained. The product was filtered and washed with fresh ethanol and air-dried. The yield was 84 g (69 percent), m.p. 160°–162°C.

B. 7-Methyl-9-imidazo[4,5-f]quinolinol

40 g of A. was added to 80 ml of boiling Dowtherm(R) and the boiling was continued for 5 minutes. The product separated upon cooling. The product was filtered, washed with Dowtherm(R) and then acetone and air-dried. The yield was 29 g (91 percent), m.p. 345°–347°C.

C. 9-Chloro-7-methylimidazo[4,5-f]quinoline

Into a 22-l, 4-necked flask set in a tub and equipped with a stirrer, an air condenser (drying tube), thermometer, and dropping funnel was placed $POCl_3$ (4590 ml). B (1062 g, 5.33 moles) was added wtih no heating effect noted. Dimethylformamide (4690 ml) was added dropwise over a 2½ hr period at a rate to control the temperature below 85°. The resulting viscous solution was allowed to stand overnight at room temperature and then added cautiously to ice to a total volume of ca. 50 **1.** The resulting solution was then adjusted to a pH of 7 to 8 by the addition of NaOHO pellets (9771 g). More icc was added as needed to keep the temperature below 45°. The resulting precipitate was collected by filtration, washed well by stirring in water (3 × 20 l) and dried at 60° to yield 1107 g (95.5 percent).

D. 9-(p-Ethylanilino)-7-methyl-1H-imidazo[4,5-f]quinoline Hydrochloride

A 500 ml, 3-neck, r.b. flask fitted with stirrer, condenser and thermometer was charged with C. (21.7 g, 0.1 mole), p-ethylaniline (12.1 g, 0.1 mole) and ethanol (200 ml). The mixture was stirred and refluxed overnight. The reaction solution was concentrated to dryness by rotary evaporator. The residue was collected and dried at 100°C to yield 34.8 g (103 percent) tan crystals, m.p. 328°–335°C. The crude material (29.3 g) was recrystallized from isopropanol (ca 1000 ml), treated with charcoal and filtered while hot. The filtrate was chilled and the crystals were collected by filtration and dried at 100°C to yield 17.7 g light yellow crystals, m.p. 327°–331°C.

Anal. Calcd. for $C_{18}H_{15}N_4$.HCl: C, 67.34; H, 5.65; N, 16.54
Found: C, 66.92; H, 5.56; N, 16.48

EXAMPLE II

9-[p-(2-Hydroxyethyl)anilino]-7-methyl-1H-imidazo[4,5-f]-quinoline Hydrochloride

A solution of 10.8 g (0.05 m) of the compound of Example I, c. and 6.8 g (0.05 m) of p-aminophenethyl alcohol in 200 ml of ethanol was heated under reflux for 18 hr. After cooling the product was filtered, washed with ethanol and recrystallized from 100 ml of H_2O with charcoal. The pale yellow crystalline product was dried to constant weight in the 60° oven, yielding 14.2 g, m.p. 300°–308°.

Calcd. for $C_{19}H_{18}N_4O$.HCl: C, 64.31, H, 5.40; N, 15.79
Found: C, 63.76; H, 5.36; N, 15.64

EXAMPLE III

9-(p-Dodecylanilino)-7-methyl-1H-imidazo[4,5-f]quinoline Hydrochloride Tetartohydrate

A 500 ml, 3-neck, r.b. flask fitted with condenser, stirrer and thermometer was charged with the compound of Example I, C. (21.7 g, 0.1 mole), p-dodecylaniline (26.1 g, 0.1 mole) and ethanol (300 ml) and the mixture was stirred overnight while heating at reflux. The reaction solution was treated with charcoal and filtered while hot. The filtrate was concentrated to dryness by rotary evaporator and the residue was collected and dried to yield 38.6 g (80.6 percent) brown crystals, m.p. 188°–195°C. A small sample (2.0 g) was dissolved in DMF (50 ml), treated with charcoal and filtered while hot and then cooled. The crystals were collected by filtration and dried at 100°C to yield 0.9 g, m.p. **213°–216°C.** Another recrystallization gave m.p. 216°–220°C.

Anal. Calcd. for $C_{30}H_{50}N_4$.HCl ¼H_2O: C, 72.02; H, 8.23; N, 11.59
Found: C, 72.05; H, 8.32; N, 11.70

EXAMPLE IV

9-[(9-Anthryl)amino]-7-mcthyl-1H-imidazo[4,5-f]quinoline

Preparation of 9-aminoanthracene:

A mixture of 9-nitroanthracene (22.3 g, 0.1 mole) and dimethylformamide (200 ml) was shaken with hydrogen over ½ teaspoon Raney active nickel catalyst in water. A hydrogen uptake of 26 psi. was recorded (calcd. 25.9 pail). The catalyst was removed by filtration and the fluorcscent filtrate was used in part B.

B. Preparation of 9-(aminoanthraceno)-7-methyl-1H-imidazo[4,5-f]quinoline

A mixture of the compound of Example I, C. (16 g, 0.0736 mole) and the filtrate from part A plus an additional 100 ml dimethylformamide was stirred overnight while heating at reflux. The solution was concentrated to about 130 ml by rotary evaporator. The solution was heated, treated with charcoal and filtered while hot. Ether (ca 400 ml) was slowly added to the filtrate and the soluton was chilled. After standing in the ice bath, thc crystals which formed which were collected by filtration and dried at 60°C to yield 22.2 g (73.5 percent). The 22.2 g was dissolved in methanol (800 ml), treated with charcoal and filtered while hot. The filtrate was concentrated to 350 ml by rotary evaporator and chilled. The crystals were collected by filtration and dired at 60°C to yield 6.3 g tan crystals, m.p. 194°–204°C.

Anal. Calcd. for $C_{28}H_{20}N_4$: C, 80.19; H, 4.84; N, 14.96
Found: C, 78.53; H, 4.71; N, 14.73

EXAMPLE V

9-(4-Chloroanilino)-7-methyl-1H-imidazol[4,5-f]quinoline Hydrochloride Hydrate

A solution of the compound of Example I, C. (44 g., 0.2 mole) and p-chloroaniline (26 g., 0.2 mole) in ethyl alcohol was refluxed for six hours. At the end of this period the solution was filtered and the solvent reduced in volume by evaporation. A solid precipitate formed and was isolated to give 76 g. Recrystallization from ethyl alcohol with charcoal gave 29 g of product.

Calcd. for $C_{17}H_{12}N_3Cl.HCl.H_2O$: C, 59.14; H, 4.09; N, 16.23
Found: C, 58.70; H, 4.24; N, 16.01

EXAMPLE VI

9-(p-Fluoroanilino)-7-methyl-1H-imidazo[4,5-f]quinoline hydrochloride

A mixture of 19.5 g (0.09 m) of the compound of Example I, C., 10 g (0.09 m) of p-fluoroaniline and 300 ml of ethanol was stirred and refluxed overnight. The solution was chilled, and the crude product collected by filtration, washed with ether and air-dried to give 22 g. After dissolving the crude product in 1000 ml of ethanol and boiling with charcoal, the filtrate was concentrated to one-half its volume, then chilled. The yield, after oven-drying (100°), was 17 g, m.p. 341°–345° with decomposition.

Anal. Calcd. for $C_{17}H_{11}FN_4.HCl$: C, 62.10; H, 4.29; N, 17.04; Cl, 10.79
Found: C, 62.56; H, 4.12; N, 17.15; Cl, 10.84

EXAMPLE VII

9-(3-Chloroanilino)-7-methyl-1H-imidazo[4,5-f]quinoline Hydrochloride

A 500 ml, 3-neck, r.b. flask fitted with stirrer, condenser and thermometer was charged with a mixture of the compound of Example I, C. (21.7 g, 0.1 mole) and p-chloroaniline (12.7 g, 0.1 mole) in dimethylformamide (200 ml). The mixture was stirred while heating at steam bath temperature overnight. The mixture was concentrated to dryness by rotary evaporator. The residue was collected and dried at 100°C to yield 30.4 g (88 percent) yellow-brown crystals, m.p. 339°–345°C. A 10 g sample was recrystallized from anhydrous methanaol (275 ml), treated with charcoal and filtered while hot. The filtrate was chilled and the crystals were collected by filtration and dried at 100°C to yield 5.5 g yellow crystals, m.p. 347°–355°C. Another recrystallization gave m.p. 351°–353°C.

Anal. Calcd. for $C_{17}H_{11}ClN_4.HCl$: C, 59.14; H, 4.09; N, 16.23
Found: C, 59.32; H, 4.08; N, 16.04

EXAMPLE VIII

9-(p-Acetamidoanilino)-7-methyl-1H-imidazo[4,5-f]quinoline hydrochloride

A mixture of 40 g (0.184 m) of the compound of Example I, C., 27.7 g (0.184 m) of p-aminoacetanilide and 500 ml of ethanol was stirred and refluxed overnight. The solution was concentrated in vacuo to give 51 g of crude product. It was then recrystallized from 3,000 ml of MeOH, with charcoal to give 42 g, m.p. 384°–387°C, with decompositon. A second recrystallization from MeOH, with charcoal, gave m.p. 381°–384°C, with decomposition.

Anal. Calcd. for $C_{19}H_{15}N_5O.HCl$: C, 62.04; H, 4.93; N, 19.04; Cl, 9.64
Found: C, 61.66; H, 4.69; N, 19.06; Cl, 9.51

EXAMPLE IX

9-[p-(N-Methylacetamido)anilino]-7-methyl-1H-imidazo[4,5-f]quinoline Hydrochloride Sesquihydrate

A. N-Methyl-4-nitroacetanilide

To 500 ml of acetic anhydride was added portionwise, 100 g (0.658) of N-methyl-p-nitroaniline. Following the addition, the solution was heated on a steam bath for 2 hrs, then stirred overnight at room temperature. The white precipitate was collected by filtration, washed with ether and air-dried to give 53 g, m.p. 153°–156°C. The filtrate was concentrated in vacuo to give another 61 g, m.p. 150°–154°C.

B. 9-[p-(N-methylacetamido)anilino]-7-methyl-1-H-imidazo[4,5-f]quinoline Hydrochloride Sesquihydrate

A mixture of 114 g (0.587 m) of N-methyl-4-nitroacetanilide (part A) and 800 ml of ethanol was shaken with hydrogen over one teaspoon of Raney active nickel catalyst in water. A pressure drop of 127 psi was recorded (calcd. 118 psi). The catalyst was removed by filtration and the ethanol filtrate refluxed overnight with 127 g (0.587 m) of the compound of Example I, C. The mixture was chilled, filtered, washed with ether and air-dried to give 115 g, m.p. 315°–318°C. The crude product was recrystallized from 4,000 ml of MeOH, with charcoal to yield 75.5 g, m.p. 315°–317°C.

room temperature. The product was filtered, washed with ethanol and air-dried to give 12 g of material. Recrystallization from 500 ml of water gave 7.5 g of pale yellow needles which darken slowly above ca 350°.

Anal. Calcd. for $C_{19}H_{18}N_6HCl·\frac{1}{2}H_2O$: C, 60.98; H, 5.12; N, 20.92
Found: C, 60.99; H, 5.04; N, 20.99

EXAMPLE XII

9-(4-Morpholinoanilino)-7-methyl-1H-imidazo[4,5-f]quinoline Hydrochloride

1. 4-Morpholinonitrobenzene (34 g, 0.163 mole) and ethanol (200 ml) were placed in a 500 ml reduction bottle. The compound was reduced with hydrogen over one-half teaspoon of Raney active nickel catalyst in water. A hydrogen uptake of 40 psi was recorded (calcd. 41.8 psi). The catalyst was removed by filtration.

2. A 500 ml, 3-neck, r.b. flask fitted with stirrer, condenser and thermometer was charged with the compound of Example I, C. (35.4 g, 0.163 mole). The above filtrate (1) was added and the mixture stirred and refluxed overnight. The reaction mixture was concentrated to dryness by rotary evaporator and the resi-

Anal. Calcd. for $C_{21}H_{21}N_5O.HCl.1\frac{1}{2}H_2O$: C, 58.75; H, 5.69; N, 17.13; Cl, 8.67
Found: C, 58.97; H, 5.32; N, 17.33; Cl, 9.27

EXAMPLE X

7-Methyl-9-(p-methylaminoanilino)-1H-imidazo[4,5-f]quinoline Dihydrochloride Monohydrate

A solution of 30 g of the compound of Example IX, in 450 ml of conc. HCl was heated under reflux for 4 hr and allowed to cool. The bright yellow crystals which separated were filtered, washed with HCl, air-dried briefly and then suspended in 300 ml of methanol and heated on the steam bath for a few minutes, during which time the yellow color disappeared. The product was filtered, washed with methanol and air dried to give 26 g as white needles which gradually decomposed in a melting point capillary to a dark, semi-liquid mass at 335°.

due was collected and dried to yield 63.9 g (99.5%) dark green crystals, m.p. 364°–374°C. A small sample (10 g) was dissolved in anhydrous methanol (800 ml), treated with charcoal and filtered while hot. Ether (ca 1,000 ml) was added until the filtrate became turbid and the mixture was chilled. The crystals were collected by filtration and dried at 100°C to yield 7.6 g green crystals, m.p. 346°–352°C. A second recrystallization gave m.p. 351°–353°C.

Anal. Calcd. for $C_{21}H_{21}N_5O.HCl$: C, 63.71; H, 5.60; N, 17.69
Found: C, 63.59; H, 5.70; N, 17.43

Anal. Calcd. for $C_{19}H_{17}N_5.2HCl.H_2O$: C, 54.83; H, 5.37; N, 17.76; Cl, 17.98
Found: C, 55.37; H, 5.25; N, 17.71; Cl, 18.31

EXAMPLE XI

9-(p-Aminoanilino)-7-methyl-1H-imidazo[4,5-f]quinoline Hydrochloride

A solution of 20 g of the compound of Example VIII in 300 ml of conc. HCl was heated under reflux for 30 min. A clear yellow solution formed after about 15 min; in another 5 min crystals began to separate. After cooling the product was filtered, washed with HCl, then with methanol and air-dried to give 20 g of yellow crystals containing a variable amount of HCl after drying in vacuo. It was therefore converted to the monohydrochloride by the following procedure. a solution of the material in 200 ml of water was made alkaline with NH_4OH. The yellow precipiate was filtered, washed with water and suspended in 300 ml of water. One equivalent (4.2 ml) of HCl was added and the mixture was heated on the steam bath, then kept overnight at

EXAMPLE XIII

9-(4-Pyrrolidinoanilino)-7-methyl-1H-imidazo[4,5-f]quinoline Hydrochloride

1. Pyrrolidino-4-nitrobenzene (27.4 g, 0.143 mole) and ethanol (200 ml) were placed in a 500 ml reduction bottle. The compound was reduced with hydrogen over one teaspoon of Raney active nickel catalyst in water. A hydrogen uptake of 33 psi was recorded (calcd. 42.3 psi). The catalyst was removed by filtration.

2. A 500 ml, 3-neck, r.b. flask fitted with stirrer, condenser and thermometer was charged with the compound of Example I, C. (31.1 g, 0.143 mole). The above filtrate (1) was added and the mixture was stirred and refluxed overnight. The reaction mixture was concentrated to dryness by rotary evaporator and the residue was collected and dried at 100°C to yield

50.6 g (93.5 percent) tan crystals, m.p. 378°-382°C. A portion (10 g) of the crude material was dissolved in anhydrous methanol (1300 ml) treated with charcoal and filtered while hot. The filtrate was concentrated by rotary evaporator to about ½ volume and then warmed. Ether was added to the warm solution until it became turbid and the mixture was then chilled. The crystals were collected by filtration and dried to yield 7.7 g yellow-brown crystals, m.p. 361°-373°C.

Anal. Calcd. for $C_{21}H_{21}N_5$.HCl: C, 66.39; H, 5.84; N, 18.44
Found: C, 65.98; H, 5.85; N, 18.17

EXAMPLE XIV

9-{4-[2-hydroxyethyl(methyl)amino]anilino}-7-methyl-1H-imidazo[4,5-f]quinoline Hydrochloride Hemihydrate

A. N-(2-Hydroxyethyl)-N-methyl-4-nitroaniline

A 500 ml, 3-neck, r.b. flask fitted with condenser, stirrer and thermometer was charged with p-nitrochlorobenzene (39.3 g, 0.25 mole) and N-methylethanolamine (37.5 g, 0.5 mole) and the mixture was heated at 160°-170°C for 3 hr. The reaction mixture was diluted with water (ca 200 ml) and stirred thoroughly. The solid was collected by filtration and recrystallized from ethanol (200 ml), treated with charcoal and filtered while hot. The filtrate was chilled and the crystals were collected by filtration and dried to yield 27 g brown crystals, m.p. 94°-97°C. A small sample (2.0 g) was again recrystallized from ethanol (25 ml), chilled, collected and dried to yield 1.1 g yellow crystals, m.p. 97°-99°C.

Anal. Calcd. for $C_9H_{12}N_2C_2$: C, 55.09; H, 6.16; N, 14.28
Found: C, 55.07; H, 6.13; N, 14.28

B. Preparation of title compound:

1. The compound of A. (23.2 g, 0.119 mole) and ethanol (200 ml) were placed in a 500 ml reduction bottle. The compound was reduced with hydrogen over one teaspoon of Raney active nickel catalyst in water and the catalyst was removed by filtration.

2. A 500 ml, 3-neck, r.b. flask fitted with stirrer, condenser and thermometer was charged with the compound of Example I, C. (25.8 g, 0.119 mole). The above filtrate 1. was added and the mixture was stirred and refluxed overnight. The reaction mixture was concentrated to dryness by rotary evaporator and the residue was collected and dried at 100°C to yield 49.1 g (107.2 percent) yellow green crystals, m.p. 299°-303°C. A portion (10 g) of the material was dissolved in methanol (200 ml), treated with charcoal and filtered while hot. Ether was added until the filtrate became turbid and was then chilled. The crystals were collected by filtration and dried at 100°C to yield 6.7 g dark green crystals, m.p. 300°-304°C.

Anal. Calcd. for $C_{20}H_{21}N_5O$.HCl ½ HCl: C, 61.13; H, 5.90; N, 17.83
Found: C, 61.48; H, 5.82; N, 17.81

EXAMPLE XV

9-(4-Morpholino-3-chloroanilino)-7-methyl-1H-imidazo[4,5-f]quinoline Hydrochloride Trihydrate

A. 3-Chloro-4-morpholinoaniline

2-Chloro-1-morpholino-4-nitrobenzene (19.5 g, 0.0806 mole) was placed in a 500 ml reduction bottle with ethanol (200 ml). The mixture was shaken with hydrogen over ½ teaspoon of Raney active nickel catalyst in water. A hydrogen uptake of 19 psi was recorded (calcd. 20.9 psi). The catalyst was removed by filtration and the filtrate was concentrated to 90 ml by rotary evaporator and chilled. The crystals were collected by filtration and dried at 60°C to yield 10.8 g off white crystals, m.p. 93°-97°C. The filtrate was further concentrated to about 10 ml and chilled. The second crop was collected and dried at 60°C to yield 3.2 g gray crystals, m.p. 92°-93°C. Total yield 14 g (81.8 percent).

Anal. Calcd. for $C_{10}H_{13}ClN_2O$: C, 56.47; H, 6.16; N, 13.17
Found: C, 56.43; H, 6.05; N, 13.19

B. Preparation of title compound:

A 500 ml, 3-neck, r.b. flask fitted with condenser, stirrer and thermometer was charged with a mixture of 3-chloro-4-morpholinoaniline (12.9 g, 0.061 mole), the compound of Example I, C. (13.2 g, 0.061 mole) and dimethylformamaide (300 ml). The mixture was heated at 100°C, with stirring, for about 2 hrs when a yellow solid formed. Heating was stopped and the mixture was stirred at room temperature overnight. The reaction mixture was then concentrated to dryness by rotary evaporator. The residue was washed with ether, collected by filtration and dried at 100°C to yield 26.9 g (100.2 percent) yellow crystals. The crude product was dissolved in methanol (800 ml), treated with charcoal and filtered while hot. Ether was added until the filtrate became turbid and was then chilled. The crystals were collected by filtration and dried at 100°C to yield 12.4 g light yellow crystals, melting 320°-327°C (dec).

Anal. Calcd. for $C_{21}H_{20}ClN_5O.3H_2O$: C, 52.07; H, 5.62; N, 14.46
Found: C, 52.19; H, 5.39; N, 14.42

EXAMPLE XVI

9-(p-Hydroxyanilino)-7-methyl-1H-imidazo[4,5-f]quinoline Hydrochloride Hemihydrate

A mixture of 33 g (0.15 mole) of the compound of Example I, C. and 15.5 g (0.15 mole) of p-aminophenol in 1,800 ml of ethanol was refluxed for 6 hr. The solvent was removed by evaporation in vacuo to leave a solid residue. The residue was recrystallized from methyl alcohol. The product was collected as tan needles melting at 390° (dec) in a yield of 24 g (48 percent).

Anal. Calcd. for $C_{19}H_{13}N_4O.HCl.½\ H_2O$: C, 60.81; H, 4.80; N, 16.69
Found: C, 60.55; H, 5.11; N, 16.71

EXAMPLE XVII

9-(m-Hydroxyanilino)-7-methyl-1H-imidazo[4,5-f]quinoline hydrochloride

A mixture of 29.9 g (0.138 m) of the compound of Example I, C., 15 g (0.138 m) of m-aminophenol and 200 ml of ethanol was stirred, and refluxed overnight. The solvent was removed by vacuum distillation to give 44 g m.p. 390° with decomposition. The crude product was recrystallized from 5,000 ml of MeOH. The yield after oven-drying (100°) was 33 g, m.p. 389°–391° with decomposition. Recrystallization from MeOH, with charcoal, gave m.p. 396°–398° with decomposition.

Anal. Calcd. for $C_{17}H_{14}N_4O.HCl$: C, 62.48; H, 4.63; N, 17.15; Cl, 10.85
Found: C, 62.18; H, 4.68; N, 16.97; Cl, 11.07

EXAMPLE XVIII

9-(3-Chloro-4-hydroxyanilino)-7-methyl-1H-imidazo[4,5-f]quinoline Hydrochloride

A stirred mixture of the compound of Example I, C., (22 g, 0.1 mole) and 3-chloro-4-hydroxyaniline (14 g, 0.1 mole) in 200 ml of dimethylformamide was refluxed for 6 hours. The reaction solution was stripped in vacuo to give 35 g (97 percent) of tan solid which when recrystallized from methanol/ether, melted at 387° dec.

Anal. Calcd. for $C_{18}H_{15}ClN_4O.HCl$: C, 56.52; H, 3.91; N, 15.51
Found: C, 56.25; H, 3.91; N, 15.29

EXAMPLE XIX

9-(4-Carbethoxyanilino)-7-methyl-1H-imidazo[4,5-f]quinoline hydrochloride

A mixture of 21.7 g (0.1 m) of the compound of Example I, C., 33 g (0.2 m) of ethyl-p-aminobenzoate and 500 ml of ethanol was stirred and heated at reflux overnight. The solution was concentrated in vacuo to dryness. The yellow semi-solid was dissolved in 500 ml of ethanol. Charcoal was added, the mixture filtered and the filtrate reduced to one-half volume. The yellow solid was collected by filtration, washed with ether and air-dried to give 23 g. Recrystallization from ethanol with charcoal, yielded 22 g, m.p. 275°–280°C.

Anal. Calcd. for $C_{20}H_{18}N_4O_2.HCl$: C, 62.74; H, 5.00; N, 14.60; Cl, 9.26
Found: C, 62.56; H, 4.88; N, 14.75; Cl, 9.47

EXAMPLE XX

9-(p-Benzoylanilino)-7-methyl-1H-imidazo[4,5-f]quinoline hydrochloride

A mixture of 6.51 g of the compound of Example I, C., 5.92 g of 4-amino-benzophenone and 150 ml of ethanol was refluxed with stirring overnight. It was then concentrated in vacuo to give 11.9 g of yellow crystals. The crude product was recrystallized from 200 ml of MeOH, with charcoal, followed by concentration of the filtrate to dryness to yield 11.2 g, m.p. 348°–350°C, after oven-drying (100°C).

Anal. Calcd. for $C_{24}H_{18}N_4O.HCl$: C, 69.48; H, 4.62; N, 13.51; Cl, 8.55
Found: C, 69.03; H, 4.68; N, 13.58; Cl, 8.45

EXAMPLE XXI

9-(5-Acetyl-2-methoxyanilino)-7-methyl-1H-imidazo[4,5-f]quinoline hydrochloride Hemihydrate

A mixture of 236 g (0.11 m) of the compound of Example I, C., 18 g (0.04 m) of 3-amino-4-methoxyacetophenone and 200 ml of ethanol was refluxed with stirring overnight. The reaction was chilled, filtered, washed with ether and air-dried to give 62 g, m.p. > 400°C.

Anal. Calcd. for $C_{20}H_{18}N_4O_2.HCl.\frac{1}{2}H_2O$: C, 61.30; H, 5.14; N, 14.64; Cl, 9.26
Found: C, 61.78; H, 5.41; N, 13.99; Cl, 8.95

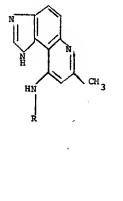
The compounds of this invention possess antibacterial qualities. They are particularly noteworthy for their ability to inhibit *Haemophilus vaginalis*, a causative agent of bacterial vaginitis. The table herebelow depicts the ability of these compounds to suppress the growth of that organism as determined using the commonly employed serial dilution technique.

Compound of Example	Minimum inhibitory concentration: in mcg./ml
I	6.25
II	25.0
III	25.0
IV	50.0
V	6.25
VI	3.1
VII	12.5
VIII	12.5
IX	6.25
X	6.25
XI	12.5
XII	25.0
XIII	12.5
XIV	25.0
XV	12.5
XVI	6.25
XVII	1.5
XVIII	6.25
XIX	25.0
XX	50.0
XXI	12.5

To provide a carrier for the compounds of this invention various pharmaceutical forms such as sprays, douches, ointments, creams and suppositories can be employed.

What is claimed is:

1. A compound of the formula:



wherein R is 4-ethylphenyl, 4-(2-hydroxyethyl)phenyl, 4-n-dodecylphenyl, 9-anthryl, 4-chlorophenyl, 4-fluorophenyl, 3-chlorophenyl, 4-acetylaminophenyl, 4-(N-methylacetylamino)phenyl, 4-methylaminophenyl, 4-aminophenyl, 4-morpholinophenyl, 4-pyrrolidinophenyl, 4-[N-methyl-N(-2-hydroxyethyl)amino]phenyl, 3-chloro-4-hydroxyphenyl, 4-carbethoxy phenyl, 3-chloro-4-morpholinophenyl, 4-hydroxyphenyl, 3-hydroxyphenyl, 4-benzoylphenyl, or 2-methoxy-5-acetylphenyl.

2. The compound of claim **1** wherein R is 4--ethylphenyl.

3. The compound of claim **1** wherein R is 4-(2-hydroxyethyl)phenyl.

4. The compound of claim **1** wherein R is 4-n-dodecylphenyl.

5. The compound of claim **1** wherein R is 9-anthryl.

6. The compound of claim **1** wherein R is 4-chlorophenyl.

7. The compound of claim **1** wherein R is 4-fluorophenyl.

8. The compound of claim **1** wherein R is 3-chlorophenyl.

9. The compound of claim **1** wherein R is 4-acetylaminophenyl.

10. The compound of claim **1** wherein R is 4-N-(methylacetylamino)phenyl.

11. The compound of claim **1** wherein R is 4-methylaminophenyl.

12. The compound of claim **1** wherein R is 4-aminophenyl.

13. The compound of claim **1** wherein R is 4-morpholinophenyl.

14. The compound of claim **1** wherein R is 4-pyrrolidinophenyl.

15. The compound of claim **1** wherein R is 4-[N-methyl-N-(2-hydroxyethyl)amino]phenyl.

16. The compound of claim **1** wherein R is 3-chloro-4-morpholinophenyl.

17. The compound of claim **1** wherein R is 4-hydroxyphenyl.

18. The compound of claim **1** wherein R is 3-hydroxyphenyl.

19. The compound of claim wherein R is 3-chloro-4-hydroxyphenyl.

20. The compound of claim **1** wherein R is 4-carbethoxyphenyl.

21. The compound of claim **1** wherein R is 4-benzoylphenyl.

22. The compound of claim **1** wherein R is 2-methoxy-5-acetylphenyl.

* * * * *

Exhibit R

US005453426A

United States Patent [19]

Jacobson et al.

[11] Patent Number: 5,453,426

[45] Date of Patent: Sep. 26, 1995

[54] **SULFUR-CONTAINING XANTHINE DERIVATIVES AS ADENOSINE ANTAGONISTS**

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[73] Assignee: **The United States of America as represented by the Department of Health and Human Services**, Washington, D.C.

[21] Appl. No.: **359,959**

[22] Filed: **Dec. 20, 1994**

Related U.S. Application Data

[63] Continuation of Ser. No. 73,654, Jun. 7, 1993, abandoned, which is a continuation of Ser. No. 340,351, Apr. 19, 1989, abandoned.

[51] **Int. Cl.**[6] **C07D 473/06**; A61K 31/52

[52] **U.S. Cl.** **514/263**; 544/267; 544/268

[58] **Field of Search** 544/267, 268; 514/263, 262

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[57] **ABSTRACT**

The present invention provides sulfur-containing xanthine derivatives which are 1,3-disubstituted with a C_1–C_{12} alkyl, which may be further substituted with a hydroxy, amino, or halo group, and are 8-substituted with either a cycloalkyl, furyl, thienyl, or substituted phenyl group. These derivatives possess increased selectivity or potency at adenosine receptors.

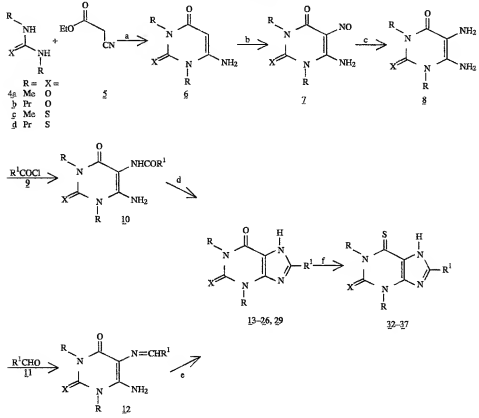
26 Claims, No Drawings

SULFUR-CONTAINING XANTHINE DERIVATIVES AS ADENOSINE ANTAGONISTS

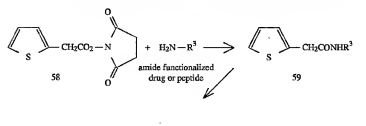
This is a continuation of application Ser. No. 08/073,654 filed on Jun. 7, 1993 now abandoned, which, in turn, is a continuation of application Ser. No. 07/340,351 filed on Apr. 19, 1989, now abandoned.

Sulfur-containing analogs of 8-substituted xanthines were prepared in an effort to increase selectivity or potency as antagonists at adenosine receptors. Either cyclopentyl- or various aryl-substituents were utilized at the 8-position, because of the association of these groups with high potency at A_1-adenosine receptors. Sulfur was incorporated on the purine ring at positions 2- and/or 6-, in the 8-position substituent in the form of 2- or 3-thienyl groups, or thienyl groups separated from an 8-aryl substituent through an amide-containing chain. The feasibility of using the thienyl group as a prosthetic group for selective iodination via its Hg^{+2} derivative was explored. Receptor selectivity was determined in binding assays using

Scheme 1.

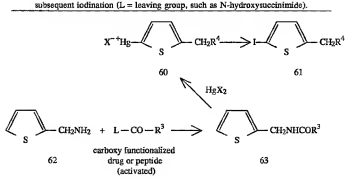


Scheme 2.
Use of 2-thienyl derivatives as prosthetic groups for mercuration and subsequent iodination (L = leaving group, such as N-hydroxysuccinimide).



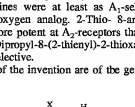
-continued

Scheme 2.
Use of 2-thienyl derivatives as prosthetic groups for mercuration and subsequent iodination (L = leaving group, such as N-hydroxysuccinimide).



membrane homogenates from rat cortex ([^{3}H]N^6-phenylisopropyladenosine as radioligand) or striatum ([^{3}H]N-ethylcarboxamidoadenosine as radioligand) for A_1- and A_2-adenosine receptors, respectively. Generally 2-thio-8-cycloalkylxanthines were at least as A_1-selective as the corresponding oxygen analog. 2-Thio- 8-aryl derivatives tended to be more potent at A_2-receptors than the oxygen analog. 1,3-Dipropyl-8-(2-thienyl)-2-thioxanthine was >285-fold A_1-selective.

Compounds of the invention are of the general formula:



wherein X is O or S, with the proviso that at least one X is S; R is alkyl of 1–12 carbons which may be substituted with hydroxy, amino, halo, or an aryl, R^1 is hydrogen, cycloalkyl, furyl, thienyl, phenyl which may be substituted with a substituent R^2 which is COOH, COOAlkyl, $CONH(CH_2)_nNHR^3$, alkoxyamid, benxyloxyamid, alkylamino; R^3 is alkyl, alkylcarbonyl, alkoxyamidoalkyl. In all cases alkyl includes cyclic, branched, or straight chain and may be substituted with halo, alkoxy, hydroxy, amino, amido, or carboxy groups.

BACKGROUND OF THE INVENTION

1,3-Dialkyl- and other xanthine derivatives inhibit many of the pharmacological and physiological effects of adenosine, eg. the cardiac depressive,[1] hypotensive,[1] anti-diuretic,[2] and anti-lipolytic effects,[3] by acting as competitive antagonists at A_1 and A_2-adenosine receptor subtypes. The naturally-occurring caffeine and theophylline, 1, are the most widely used xanthine drugs. However, they are non-selective and relatively weak adenosine antagonists (K_i-values of 10 μM or greater). Synthetic analogs of theophylline, containing 1,3-dipropyl-, 8-aryl, and 8-cycloalkyl-substitutions, are more potent as adenosine antagonists.[4-6] The combination of 1-, 3-, and 8-position substitutions has resulted in analogs such as CPX,[5,6] 2, and XAC,[7] 3, which are more than four orders of magnitude more potent than theophylline in binding at A_1-adenosine receptors, and A_1-selective by factors of 300 and 60, respectively.

Xanthines having thio-substitutions at the 2- and/or 6-position have been reported to act as antagonists as A_2-receptors in human fibroblasts[21] and as phosphodiesterase inhibitors with potency comparable to or greater than theophylline.[8,9a] Remarkably, 6-thiocaffeine and 6-thiotheophylline cause cardiac depression rather than stimulation.[9b] Recently, 6-thiocaffeine and 6-thiotheophylline were reported to induce tracheal relaxation, without cardiac or behavioral stimulation.[9a] Thio-substitution of the NH at position 7 of 8-phenyltheophylline reduced activity by 1000-fold at an A_1 receptor and by nearly 100-fold at an A_2 receptor.[20]

DESCRIPTION OF THE INVENTION

The compositions of the invention provide means of increasingly selective acitivity as adenosine receptor antangonists in xanthine drugs.

Various 8-substituted xanthine and thioxanthine derivatives were synthesized via 1,3-dialkyl-5,6-diaminouracils as shown in Scheme 1. The substituted uracil and 2-thiouracil intermediates were prepared via an optimized Traube synthesis.[6,7a] 1,3-Dimethyl- and 1,3-di-n-propyl-5,6-diaminouracil and their 2-thio derivatives were obtained by condensation of the corresponding dialkyl urea, 4a and b, or thiourea, 4c and d, with ethyl cyanoacetate, 5. The products after ring closure, substituted 6-aminouracil derivatives, 6, were then nitrosated at the 5-position. The nitroso group was reduced through chemical reduction or catalytic hydrogenation to form the intermediate 1,3-dialkyl-5,6-diaminouracil derivatives, 8, in good yield.

The next step of the synthesis was to form the imidazole ring of the purine nucleus resulting in the xanthine derivatives, as listed in Table 1 (compounds 1,2, 13–28). The more nucleophilic 5-amino group of compound 8 was acylated using a carboxylic acid chloride, 9, forming the 1,3-dialkyl-5-acylamino- 6-aminouracil derivatives, 10. 1,3-Dialkyl-5-acylamino-6-aminouracils derived from thiophene-2-carboxylic acid and -3-carboxylic acid chlorides and from cyclopentane carboxylic acid chloride were isolated and characterized (Table 2). The various 1,3-dialkyl-5-acylamino-6-aminouracil derivatives were then cyclized to the corresponding xanthine and 2-thioxanthine derivatives (Table 3) by treatment with aqueous sodium hydroxide.

For 8-(p-carboxymethyloxyphenyl)xanthine derivatives related to a xanthine amine congener, compound 3, an

alternate route was used to form the imidazole ring. 1,3-Dipropyl-5,6-diaminouracil, 8b, or corresponding 2-thiouracil, 8d, was condensed with p-formylphenyloxyacetic acid, forming the imine, 12. Upon oxidation, the carboxylic acid congeners 27 (XCC) and 28 were obtained. The xanthine carboxylic acid derivatives were then esterified giving the ethyl esters, 29 and 30, respectively, which were treated with neat ethylene diamine as previously reported[7] to give the amine derivatives 3 and 31. Since the A_2- potency of compound 31 was enhanced over the oxygen analog (see below), compound 3, we synthesized other 8-aryl-2-thioxanthine derivatives in an effort to increase A_2-potency. Other amine derivatives were synthesized through aminolysis reactions (compounds 32–35) or by carbodiimide coupling (compounds 36–39) as reported.[10e] Lysyl conjugates 38–43 were prepared as described.[10e]

An N-hydroxysuccinimide ester derivative, 44, was reported to be an irreversible inhibitor of A_1-adenosine receptors at concentrations greater than 50 nM.[10a] If shown to be potent and non-selective adenosine antagonist this xanthine may be a potential inhibitor of both A_1 and A_2-adenosine receptors. Certain isothiocyanate-containing xanthines related to compound 3 also have been shown to be chemically reactive with A_1-receptors.[10a] Efforts to synthesize analogous xanthineisothiocyanates containing the 2-thio subsiuition were unsuccessful, likely due to side reactions involving the more reactive thio group.

A thiation reaction was used to generate 6-thioxanthine derivatives from the corresponding oxygen analogs. It is known[11] that xanthine derivatives are preferentially thiated at the 6-position using P_4S_{10}. Dioxane was the favored reaction medium to give high yields of the anticipated 6-thio- and 2,6-dithioxanthines (compounds 52–57). For example, CPX was converted to 8-cyclopentyl-1,3-dipropyl-6-thioxanthine, 55, using this thiation reaction.

Iodinated xanthine derivatives, synthesized using a prosthetic group[12] or by classical methodology, have been introduced as high affinity radioligands for adenosine receptors.[12,13] We have explored the use of a 2-thienyl substituent as a site for selective iodination, via mercuration (Scheme 2). These substituted thiophene derivatives such as 59 and 63 undergo regioselective mono-mercuration at the unsubstituted 2-position, rapidly and at ambient temperature, in the presence of stoichiometric quantities of mercury salts such as mercuric acetate.[14] The 2-mercurothiophene salt, 60, is then exposed to elemental iodine resulting in the corresponding 2-iodothiophene derivative, 61.

New prosthetic groups designed for facile radiodination of functionalized drugs and peptides are still being sought.[15,16] We have used thiophene-2-acetic acid (as its reactive N-hydroxysuccinimide ester, 58, and thiophene-2-methylamine, 62, as prosthetic groups for iodination, via mercuration.

Compound 58 reacted with XAC, 3, to form an amide, compound 46. This 2-thienyl xanthine was readily mercurated to give 47.

Iodination via 2-mercurothiophene intermediates as in Scheme 2 may be carried out selectively in the presence of other susceptible aromatic groups, such as phenols. Compound 58 reacted with L-tyrosyl-glycine to form an amide (compound 59, in which R^3=CH(CH$_2$$\phi$OH)CONHCH$_2$COOH). Upon sequential treatment with mercuric acetate and iodine (1 equiv.), the corresponding monoiodinated peptide derivative, 61 (R^3= CONHCH(CH$_2$$\phi$OH)CONHCH$_2$COOH), was obtained in high yield.

The N-succinoyl derivative (63a, R^2=(CH$_2$)$_2$COOH) of thiophene-2-methylamine was mercurated to form an internal salt, 60b (R^4=NHCO(CH$_2$)$_2$COOH) which precipitated from methanol. Upon treatment with iodine an immediate reaction occurred. This reaction was followed by NMR in d_6-DMSO. The complete reaction of the 2-mercurothiophene derivative was indicated by shifts of the thiophene aromatic signals to 6.68 and 7.13 ppm from TMS, corresponding to the 2-iodo derivaiive, 61b.

Results—Pharmacology

Affinity at A_1- and A_2-adenosine receptors was measured in competitive binding assays, using as radioligands [^{3}H]N^6-phenylisopropyladenosine [17] (with rat cerebral cortical membranes) and [^{3}H]5'-N-ethylcarboxamidoadenosine (with rat striatal membranes), [18] respectively.

A sulfur substituiion at the 2-position carbonyl group of 1,3-dialkylxanthines usually did not decrease the affinity of the xanthines for A_1 or A_2 adenosine receptors. In the case of the 2-thio analog of CPX, compound 14, the A_1 affinity was enhanced by the thio substitution. The 2-thio xanthine amine congener, compound 30, bound with greater affinity at A_2-receptors and with less affinity at A_1-receptors than the corresponding oxygen analog, compound 3. Potency at A_2-receptors was enhanced 7-fold by the 2-thio substitution in the case of a carboxylic acid congener (compounds 27 and 28).

N-Methylated analogs (32–37) of compound 31 were prepared. As in the 2-oxo series, the secondary N-methylamine derivative, 33, was the most potent at A_2-receptors with a K_i-value of 6.8 nM. Thus, the combination of two modifications of compound 3 have enhanced its A_2 affinity 10-fold.

A sulfur substitution at the 6-position carbonyl group of 1,3-dialkylxanthines was not well tolerated at either A_1 or A_2 binding sites. Thus, the 6-thio analog of CPX, 55, was 17-fold less potent than CPX at A_1-receptors. The 6-thio analog of 1,3-diethyl- 8-phenylxanthine, 57, was 23-fold less potent than DPX, 24, at A_1-receptors and 12-fold less potent at A_2-receptors. 2,6-Dithio analogs, such as 53, were intermediate in potency between the corresponding 2-thio and 6-thio analogs.

Substitutions of thienyl and furyl groups at the 8-position of xanthines have approximately equivalent effects on affinity at both receptor subtypes. Both substitutions are generally slightly less potent than the corresponding 8-phenylxanthine analogs. For both thienyl and furyl derivatives (several of which were reported previously, by Bruns et al[18]), attachment at the 3-position relative to the heteroatom (sulfur or oxygen, respectively) results in greater potency at adenosine receptors than attachment at the 2-position. Preference for 3-thienyl derivatives was evident particularly at the A_2-subtype (cf. 18 vs. 17 and 20 vs. 19).

1,3-Dipropyl-8-(2-thienyl)-2-thioxanthine, 22, did not bind measurably to A_2-receptors at its limit of solubility (approximately 10 μM), at which concentration there was not even a partial displacement of tritiated [^{3}H]N-ethylcarboxamidoadenosine from striatal membranes. Thus, compound 22 is >285-fold A_1-selective in these binding assays.

2-Thio analogs of caffeine were also prepared. 8-Cycloalkyl- 2-thio analogs (eg. cyclohexyl and cyclopentyl) of 1,3,7-trialkylxanthines are A2 selective adenosine antagonists. When 8-aryl substituents are present, the caffeine analogs tend to be more non-selective than the corresponding 8-cycloalkyl derivative.

The following compounds were prepared as caffeine analogs:

Compound

64 8-Cyclopentyl-2-thiocaffeine

65 8-(2-Thienyl)-2-thiocaffeine

66 8-(2-Thienyl)caffeine

Discussion

We have found that 2-thioxanthines are very similar in potency to the corresponding oxygen analogs. In certain cases, as for the CPX analog, 14, and an 8-(2-thienyl) derivative, 22, a greater margin of A_1-selectivity may be achieved using the 2-thio substitution. A 6-thio substiution is not well tolerated at either A_1 or A_2 receptors.

The feasibility of using a 2-thienyl moiety as a prosthetic group for selective iodination via its $HgCl^{+3}$ derivative was explored. By facile and selective mercuration at the 2-thienyl Ar—H, a site for rapid and regioselective (in the presence of phenols) iodination is created. A xanthine conjugate of XAC and 2-thienylacetic acid was sequentially mercurated and iodinated by this scheme, resulting in an iodinated xanthine with potential use as an antagonist radioligand for adenosine receptors. This scheme may have applicability to other receptor ligands, including tyrosyl peptides, in which iodination in the presence of essential phenolic groups, is desired.

Experimental Section

XAC (8-(2-aminoethylaminocarbonylmethyloxyphenyl-1,3-dipropylxanthine), 2-chloroadenosine, and 8-cyclohexyl-1,3-dipropylxanthine were obtained from Research Biochemicals, Inc. (Natick, Mass.). Compounds 32, 34, 36, 38, 42, 50, and 51 were reported previously.[9c,10b] Amino acid derivatives of XAC and the 2-thio analog were synthesized in the manner previously described,[9c] using the water soluble 1-(3-dimethylaminopropyl)- 3-ethylcarbodiimide hydrochloride (EDAC) in dimethylformamide. p-Formylphenyloxyacetic acid was obtained from Eastman-Kodak (Roehester, N.Y.). [^{3}H]N^6-phenylisopropyladenosine and [^{3}H]5'-N-ethylcarboxamidoadenosine were from Dupont NEN Products, Boston, Mass. Thiophene-2-acetic acid was from Aldrich.

New compounds were characterized by 300 MHz proton NMR (unless noted chemical shifts are in d_6-DMSO in ppm from TMS), chemical ionization mass spectroscopy (CIMS, NH_3, Finnigan 1015 spectrometer), and C, H, and N analysis. UV spectra were measured in methanol, and the results are expressed as peak wavelengths in nm with log ε values in parentheses.

General procedure for compound 10

A 1,3-disubstituted 5,6-diaminouracil or the corresponding 1-thiouracil (10 mmol) was suspended in 10 ml of absolute pyridine and then under stirring 11 mmol of the acid chloride (freshly prepared) was added dropwise. After 5 h stirring at room temperature the reaction mixture was poured slowly into 100 ml of H_2O, and the precipitate was collected by suction filtration. Purification was done by recrystallization from a EtOH/H_2O-mixture. Yields ranged from 70 to 90%.

General procedure for compounds 13–26

A 1,3-disubstituted 5-acylamino-6-aminouracil or the corresponding 2-thiouracil (10 mmol) was heated under reflux in a mixture of 40 ml of 1N NaOH and 10 ml of EtOH for 1 h. The hot solution was acidified with acetic acid resulting in the formation of a precipitate upon cooling. The precipitate was collected and recrystallized from a H_2O/EtOH-mixture. Yield 80–90% of colorless crystals. ^{1}H-NMR spectrum of compound 13: δ 3.86 (3H, s, CH_3), 3.68 (3H, s, CH_3), 3.19 (m, 1H, cyclohex C1), 2.0 (m, 2H, cyclohex C2 and C5), 1.6–1.8 (m, 6H, cyclohex). The NMR spectra of the other compounds were consistent with the assigned structures.

(8-[2-Dimethylaminoethyl[N-methylamino[carbonyl[methyl[oxyphenyl]]]]]-2-thio-1,3-dipropylxanthine), 37

Compound 28, 8-(4-(carboxymethyloxy)phenyl)-1,3-dipropyl-2-thioxanthine (21 mg, 52 μmol), N,N,N'-trimethylethylenediamine (Aldrich, 20 mg, 0.20 mmol), EDAC (45 mg, 0.23 mmol), and N-hydroxybenzotriazole (25 mg, 0.18 mmol) were combined in 1 mL DMF. After stirring overnight, 0.5 mL of sodium carbonate (pH 10, 0.5M) and 2 mL of saturated NaCl were added. After cooling overnight a white precipitate was collected. Yield 13 mg (52%). The NMR and mass spectra were consistent with the assigned structure.

N-Succinimidyl 8-[2-[4-(6-Carboxy-n-hexyl)carbonylaminoethyl[amino[carbonyl[methyloxyphenyl]]]]-1,3-dipropyl-2-thioxanthine, 45

Compound 31 (10.4 mg, 0.024 mmol) was added to a solution of disuccinimidyl suberate (13.1 mg, 0.036 mmol, Pierce, Rockford, Ill.) in DMF (1 mL) and vigorously stirred for 2 h or until complete by TLC (CHCl3/MeOH/AcOH, 18/1/1). Dry ether (2 mL) was then added to the suspension followed by the addition of petroleum ether until cloudy. The suspension was allowed to stand at 0° C. for 1 h then filtered to give an off-white powder. Yield 11.5 mg, (68%). Mp 160°–168° C. ^{1}H-NMR (DMSO-d6) 0.90 (t, J=7.2 Hz, 3 H) 0.94 (t, J=7.2 Hz, 3 H), 1.28 (br m, 4 H), 1.44 (m, 2 H), 1.71 (m, 2 H), 1.83 (m 2 H), 1.83 (t, J=7.6 Hz, 2 H), 2.65 (t, J= 7.0 Hz, 2 H), 2.79 (s, 4 H), 3.15 (br s, 4 H), 3.32 (s, H_2O), 4.45 (br t, J=7.2 Hz, 2 H), 4.54 (s, 2 H), 4.59 (br t, J=7.2 Hz, 2 H), 7.10 (d, J=8.7 Hz, 2 H), 7.83 (br s, 1 H), 8.11 (d, J= 8.7 Hz 2 H), 8.18 (br s, 1 H).

General procedure for thiation reaction

The appropriate xanthine or 2-thioxanthine derivative (10 mmol) was heated with 6 g of P_4S_{10} in 100 ml of dioxane for 3–5 h under reflux. Insoluble material was removed by filtration, and the filtrate was added dropwise to 200 ml of H_2O with stirring. The precipitate was collected and purified by recrystallization from a H_2O/EtOH-mixture. Yield 80–85%.

N-Succinimidyl thiophene-2-acetic acid, 58

Thiophene-2-acetic acid (1.61 g, 11 mmol), dicyclohexylcarbodiimide (2.34 g, 11 mmol), and N-hydroxysuccinimide (1.30 g, 11 mmol) were added to 50 mL of ethyl acetate containg 10% DMF. After stirring for two hours, the urea was removed by filtration. The filtrate was washed with aqueous acid/base and evaporated. The residue was recrystallized from ethyl acetate/petroleum ether. Yield 2.01 g (74%).

5-Mercurothiophene-2-acetate, 60a (R^4—COO^{-+}), mercuration reaction

Thiophene-2-acetic acid (0.39 g, 2.8 mmol) was dissolved in 8 mL methanol. Mercuric acetate was added with stirring, and a white precipitate appeared shortly thereafter. After one hour, 4 mL ether was added and the solid was collected by filtration. Yield of 60a 0.88 g (93%). Mass spectrum (CI-NH_3) shows a peak at 360 z/e corresponding to m+1+NH_3.

The thiophene-xanthine derivative, 46, was prepared as reported previously. Upon mercuration in dimethylformamide by a method, a solid product, compound 46, was obtained and characterized by californium plasma desorption mass spectoscopy.

2-(N-Succinoylaminomethyl)-5-mercurothiophene, 60b (R=NHCO$(CH_2)_2COO^{-+}$)

Compound 63a (60.5 mg, 0.28 mmol) was dissolved in 5 mL methanol and treated with 100 mg (0.31 mmol) mercuric acetate. First a solution formed, followed by crystallization of product. After one hour, ether was added, and the precipitate was collected. Yield 98 mg (84%), m.p. 230° C. dec.

This compound was converted to the corresponding 5-iodo derivative upon treatment at room temperature with iodine or iodine monochloride. H-NMR of 2-(N-succinoyl-laminomethyl)-5-mercuriothophene, 61b: 8.46 (1H, t, NH), 7.13 (1H, d, Ar-4), 6.68 (1H, d, Ar-3), 4.37 (2H, d, CH_2N), 2.43 (2H, CH_2), 2.35 (2H, CH_2).

5-Iodothiophene-2-acetic acid, 61a (R^4—COOH)

Compound 60a (75 mg, 0.22 mmol) was suspended in 5 mL of dimethylformamide containing 5% DMSO. Iodine crystals (77 mg, 0.31 mmol) was added with stirring. A solution formed within one minute. 1M Hydrochloric acid was added and the mixture was extracted three times with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and evaporated. The product was purified by column chromatography on silica gel. R_f of product (silica, ethyl acetate/petroleum ether) was 0.79.

The identical product was obtained as follows: N-Iodosuccinimide (236.2 mg, 1.05 mmol) was added to a stirred suspension of compound 59a (326.2 mg, 0.95 mmol) in methanol (30 mL). After 16 h the suspension was filtered and the methanol removed in vacuo. The remaing oil was redissolved in ethyl acetate, washed with 0.5N HCl and the product was extracted into a 0.5N NaOH solution. The basic fraction was washed with CH_2Cl_2, acidified to pH 1.0 with 1N HCl, and extracted with EtOAc. The product was chromatographed on a silica gel column (eluent, 17/2/1, $CHCl_3$/MeOH/AcOH), and the solvents removed from the product fractions in vacuo. Acetic acid was removed by azeotropic distillation with petroleum ether. The light yellow oil was redissolved in ethyl acetate, and ether was added forming a precipitate which was removed by filtration. Evaporation of the solvent gave compound 61a as a waxy yellow solid (110 mg, 43%).

Peptide derivatives of thiophene-2-acetic acid

Compound 58 reacted with L-tyrosyl-glycine (274 mg, 1.15 mmol) in dimethylformamide to give N-(thiophene-2-acetyl)-L-tyrosyl-glycine (230 mg, 55% yield). Upon mercuration in dimethylformamide as for compond 60a, N-(5-mercuriothiophene-2-acetyl)-L-tyrosyl-glycine (40% yield) was obtained.

2-(N-Succinoylaminomethyl)-thiophene, 63a ($R=(CH_2)_2COOH$)

Thiophene-2-methylamine (Aldrich, 2.37 g, 21 mmol) was dissolved in 20 mL tetrahydrofuran and treated with a solution of succinic anhydride (2.1 g, 21 mmol) in 20 mL dimethylformamide. After one half hour, ethyl acetate (50 mL) was added, and the mixture was extracted with citric acid (1M) three times and with water. The organic layer was dried ($MgSO_4$). Solvent was removed and petroleum ether was added causing white crystal of 62a to precipitate. Yield 9,4%, mp 130° C. The NMR and mass spectra were consistent with the assigned structure. UV spectrum λ_{max} 233 nm ($\log \epsilon$ 4.019).

Biochemical assays

Stock solutions of xanthines were prepared in the millimolar concentration range in dimethylsulfoxide and stored frozen. Solutions were warmed to 50° prior to dilution in aqueous medium. Inhibition of binding of 1 nM [^{3}H]N^6-phenylisopropyladenosine to A_1 adenosine receptors in rat cerebral cortical membranes was assayed as described.[17] Inhibition of binding by a range of concentrations of xanthines was assessed in triplicate in at least three separate experiments. IC_{50} values computer-generated using a non-linear regression formula on the Graphpad program, were converted to K_i values using a K_D value for [^{3}H]PIA of 1.0 nM and the Cheng-Prusoff equation.[19]

Inhibition of binding of [^{3}H]N-ethylcarboxamidoadenosine to A_2-adenosine receptors in rat striatal membranes was measured as described,[18] except that 5 mM theophylline was usecl to define non-specific binding. N^6-Cyclopentyladenosine was present at 50 nM to inhibit binding of the ligand at A_1-adenosine receptors. Inhibition of binding by a range of concentrations of xanthines was assessed in triplicate in at least three separate experiments. IC_{50}-values were converted to Ki-values by the method of Bruns et al,[18] using a conversion factor derived from the affinity of [^{3}H]N-ethylcarboxamidoadenosine at A_2 receptors and the Cheng-Prusoff equation.[19]

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Abbreviations: XAC, xanthine amine congener, 8-(4-(2-amino-ethylaminocarbonylmethyloxy)phenyl)- 1,3-dipropylxanthine; CPX, 8-cyclopentyl-1,3-dipropylxanthine; EDAC, dimethylaminopropylethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole.

Pharmaceutical compositions may be prepared using the usual carriers and may be given by mouth, parenterally, or as compositions particularly adapted for uptake though the mucosa, such as cyclodextrin inclusion complexes and the like. Compositions particularly suited for oral dosing include elixirs and tablets. Dosage will depend on the size of the patient and the particular preparation given. An effective dosage of 0.1 to 10 mg/kg would be the usual dosage range.

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TABLE 1

Compound	R	R^1	X	K_i A_1-receptors	K_i A_2-receptors	$K_i(A_2)/K_i(A_1)$
	Potencies of xanthine derivatives at adenosine A_1 and at A_2 receptors in nanomolar concentration units.[a,b]					
1a	Me	H	O	8,470 ± 1490[c]	25,300 ± 2000[c]	2.99
1b	Pr	H	O	450 ± 25[c]	5,160 ± 590	11.5
2a	Me	cyclopentyl	O	10.9 ± 0.9[c]	1,440 ± 70[c]	133
2b	Pr	cyclopentyl	O	0.9 ± 0.1	420	470
13	Me	cyclopentyl	S	8	1,390 ± 88	170
14	Pr	cyclopentyl	S	0.655 ± 0.058	314 ± 62	479
15	Me	2-furyl	O	350 ± 20[c]	2,780 ± 50[c]	7.94
16	Me	3-furyl	O	72.4 ± 3.7[c]	984 ± 70[c]	13.6
17	Me	2-thienyl	O	233 ± 48.6	1,630 ± 179	6.97
18	Me	3-thienyl	O	152 ± 27	841 ± 109	5.53
19	Pr	2-thienyl	O	16.1 ± 1.96	381 ± 27.7	23.6
20	Pr	3-thienyl	O	10.0 ± 0.03	121 ± 18.2	12.1
21	Me	2-thienyl	S	221 ± 43.3	1,740 ± 153	7.87
22	Pr	2-thienyl	S	35.1 ± 6.0	>10,000	>285
23	Me	phenyl	O	86.0 ± 2.8[c]	848 ± 115[c]	9.85
24	Et	phenyl	O	44.5 ± 1.2[c]	836 ± 73[c]	19.4
25	Pr	phenyl	O	10.2 ± 2.6[c]	180 ± 29[c]	17.8
26	Me	phenyl	S			
	R^2					
27	COOH		O	58 ± 3	2,200 ± 526	37.8
28	COOH		S	53.8 ± 7.1	315 ± 60.8	5.86
29	$COOEt_2$		O	42 ± 3	>10,000	>238
30	$COOEt_2$		S	6.78 ± 0.64	>5,000	>740
3	$CONH(CH_2)_2NH_2$		O	1.2 ± 0.5	63 ± 21	52.5
31	$CONH(CH_2)_2NH_2$		S	2.69 ± 0.77	26.3 ± 1.76	9.8
32	$CONH(CH_2)_2NHCH_3$		O	15.1 ± 1.6[c]	[9.3 ± 2.1][c]	[0.62]
33	$CONH(CH_2)_2NHCH_3$		S	2.4 ± 0.28	6.80 ± 1.36	2.8
34	$CONH(CH_2)_2N(CH_3)_2$		O	2.8 ± 0.19[d]	5.03 ± 0.54	1.8
35	$CONH(CH_2)_2N(CH_3)_2$		S	2.55 ± 0.60	27.9 ± 7.5	11
36	$CONCH_3(CH_2)_2N(CH_3)_2$		O	0.93 ± 0.03[d]	6.26 ± 0.25	6.7
37	$CONCH_3(CH_2)_2N(CH_3)_2$		S	2.57 ± 0.67	24.5 ± 8.4	9.5
38	$CONH(CH_2)_2NHCOCHNHCOOBu^t$ (D) $(CH_2)_4$ $C_6H_5CH_2OCONH$		O	12	e	e
39	$CONH(CH_2)_2NHCOCHNHCOOBu^t$ (D) $(CH_2)_4$ $C_6H_5CH_2OCONH$		S	84	870	10
40	$CONH(CH_2)_2NHCOCHNH_2$ (D) $CF_3COOH.$ $(CH_2)_4$ $C_6H_5CH_2OCONH$		O	6.4 ± 2.7	191 ± 13	30
41	$CONH(CH_2)_2NHCOCHNH_2$ (D) $CF_3COOH.$ $(CH_2)_4$ $C_6H_5CH_2OCONH$		S	8.9	322 ± 17	36
42	$CONH(CH_2)_2NHCOCHNH_2$ (D) $2HBr.$ $(CH_2)_4$ NH_2		S	0.87 ± 0.09	180	210

TABLE 1-continued

Potencies of xanthine derivatives at adenosine A_1 and at A_2 receptors in nanomolar concentration units.[a,b]

Compound		X	K_i A_1-receptors	K_i A_2-receptors	$K_i(A_2)/K_i(A_1)$
43	$CONH(CH_2)_2NHCOCHNH_2$ (D) 2HBr. $(CH_2)_4$ NH_2	S	13.0 ± 3.5	46.8 ± 9.4	3.6
44	$CONH(CH_2)_2NH-CO(CH_2)_4COOH$	O	3.69 ± 0.71[d]	207 ± 57[d]	56
45	$CONH(CH_2)_2NH-CO(CH_2)_4COOH$	S	33.5	e	e
46	$CONH(CH_2)_2NH-COCH-$	O	18.3 ± 3.0	147 ± 5	8.1
47	$CONH(CH_2)_2NH-COCH_2-HgOAc$	O	16.2 ± 2.7	458 ± 34	28.3
48	$CONH(CH_2)_2NH-COCH_2-I$	O	11.3 ± 1.5	116 ± 25	10.3
49	$CONH(CH_2)_2NH-CO(CH_2)_6CONHCH_2-$		7.44 ± 0.98	630 ± 160	85
50	$CONH(CH_2)_2NHCOCHNHCOO-Bu^t$ CH_2 (L)	O	17.6 ± 1.6	e	e
51	$CONH(CH_2)_2NHCOCHNH_2$ (L) $CF_3COOH.$ CH_2	O	1.3 ± 0.12	e	e
	R / R^1				
52	Me / cyclopentyl	S	40.5 ± 6.6	11,500 ± 628	285
53	Pr / cyclopentyl	S	4.87 ± 0.82	2,780 ± 730	572
54	Me / cyclopentyl	O	202 ± 26	8,930 ± 1300	44.4
55	Pr / cyclopentyl	O	15.5 ± 1.5	3,360 ± 270	217
56	Me / phenyl	O	1,380 ± 74	11,300 ± 777	8.18
57	Et / phenyl	O	1,010 ± 321	3,510 ± 290	3.47

[a] - K_i value from a single determination run in triplicate or average of thre ± S.E.M.

[b] - Inhibition of binding of [^{3}H]-phenylisopropyladenosine to A_1-receptors i rat cortical membranes and binding of [^{3}H]N-ethylcarboxamidoadenosine to A_2 adenosine receptors in rat striatal membranes was measured as described.[18,19]

[d] - Values taken from Bruns et al.[18]

[e] - Values taken from Jacobson et al.[10]

[f] - Not determined.

[g] - K_i for inhibition of N-ethylcarboxamidoadenosine-stimulated adenylat cyclase, pheochromocytoma PC12 cell membranes.[10b]

TABLE 2

Synthesis and characterization of 1,3-dialkyl-5-acylamino-6 aminouracils.

Compound	R	R^1	X	% Yield	Mp (°C.)	Formula	Analysis
10a	Me	cyclopentyl	S	71	253	$C_{13}H_{18}N_4O_2S$	C, H, N
10b	Pr	cyclopentyl	S	92	103	$C_{14}H_{36}N_4O_2S.H_2O$	C, H, N
10c	Me	2-thienyl	O	76	>300	$C_{11}H_{12}N_4O_2S$	C, H, N
10d	"	3-thienyl	O	78	>300	$C_{11}H_{12}N_4O_2S$	C, H, N
10e	Pr	2-thienyl	O	88	143	$C_{14}H_{18}N_4O_2S$	C, H, N
10f	"	3-thienyl	O	88	144	$C_{14}H_{18}N_4O_2S$	H, N; C[a]
10g	Me	2-thienyl	S	83	>300	$C_{11}H_{12}N_4O_2S_2$	C, H, N
10h	Pr	"	S	80	150	$C_{13}H_{18}N_4O_2S_2$	C, H, N

a - Calculated 53.56% C, found 52.95%.

TABLE 3

Synthesis and characterization of xanthine derivatives.

Compound	% Yield	Mp (°C.)	Formula	Analysis
13	91	278	$C_{25}H_{19}N_5OS$	C, H, N
14	89	217	$C_{16}H_{28}N_4OS$	C, H, N
17	82	>300	$C_{11}H_{10}N_4O_2S$	C, H, N
18	92	>300	$C_{11}H_{10}N_4O_2S$	C, H, N
19	85	259	$C_{25}H_{19}N_5O_2S$	C, H, N
20	87	267	$C_{25}H_{19}N_5O_2S$	C, H, N
21	71	>340	$C_{11}H_{10}N_4O_2S_2$	C, H, N
22	92	298	$C_{25}H_{19}N_5OS_2$	C, H, N
26	—	350	$C_{25}H_{19}N_5OS$	C, H, N
33	95	206–208	$C_{25}H_{19}N_5O_2S.1/4H_2O$	C, H, N
35	93	238–240	$C_{25}H_{19}N_5O_2S.3/4H_2O$	C, H, N
37	52	172–174	$C_{25}H_{19}N_5O_2S.1/2H_2O$	C, H, N
39	92	210–212	$C_{40}H_{25}N_5O_2S$	C, H, N
41	84	182–187	$C_{93}H_{47}F_3N_{10}O_4S.1/2CF_3COOH.1/2H_2O$	C, H, N
43	97	238–242d	$C_{25}H_{19}N_5O_2S.3HBr.3/2H_2O$	C, H, N
45	68	160–168	$C_{25}H_{19}N_5O_2S$	C, H; N[b]
48	85	240d	$C_{29}H_{17}N_5O_2SI.2.5H_2O$	C, H, N
52	85	236	$C_{25}H_{19}N_5S_2$	C, H, N
53	84	135	$C_{25}H_{23}N_5S_2$	—
54	79	241	$C_{25}H_{19}N_5OS$	C, H, N
55	81	153	$C_{30}H_{29}N_5OS$	C, H, N
56	84	256	$C_{35}H_{27}N_5OS$	C, H, N
57	72	223	$C_{35}H_{29}N_5OS$	H; C, N[a]
60b	84	230	$C_{11}H_6NO_3SHg$	C, H, N
61a	43	71–73	$C_6H_3O_2SI.0.5H_2O$	C, H

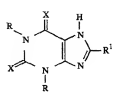
a - Calculated 59.32% C, 18.65% N; found 58.52% C, 18.12% N.
b - Calculated 14.05% N; found 15.42% N.

Supplementary information:

Compound	Formula	Elemental Analyses: Calculated % C	Calculated % H	Calculated % N	Found % C	Found % H	Found % N
10a	$C_{25}H_{19}N_5OS$	51.10	6.42	19.84	51.15	6.48	19.89
10b	$C_{45}H_{38}N_8O_2S.H_2O$	53.92	7.90	15.71	53.96	7.80	15.93
10c	$C_{11}H_{12}N_4O_2S$	47.14	4.32	20.00	47.15	4.20	19.82
10d	$C_{11}H_{12}N_4O_2S$	47.14	4.32	20.00	47.16	4.26	19.91
10e	$C_{12}H_{20}N_4O_2S$	53.56	5.99	16.65	53.60	6.10	16.46
10f	$C_{12}H_{20}N_4O_2S$	53.56	5.99	16.65	52.95	6.06	16.43
10g	$C_{11}H_{12}N_4O_2S_2$	44.58	4.08	18.90	44.43	4.24	18.86
10h	$C_{25}H_{20}N_4O_2S_2$	51.13	5.72	15.90	50.92	5.59	15.75
13	$C_{25}H_{19}N_5OS$	54.52	6.10	21.20	54.44	6.35	21.25
14	$C_{16}H_{28}N_4OS$	59.99	7.55	17.50	59.97	7.68	17.58
17	$C_{11}H_{10}N_4O_2S$	50.40	3.84	21.36	50.43	3.79	21.32
18	$C_{11}H_{10}N_4O_2S$	50.40	3.84	21.36	50.29	3.88	21.30
19	$C_{25}H_{19}N_5O_2S$	56.60	5.69	17.60	56.14	5.66	17.25
20	$C_{25}H_{19}N_5O_2S$	56.60	5.69	17.60	56.10	5.69	17.27
21	$C_{11}H_{10}N_4O_2S_2$	47.46	3.62	20.13	47.72	3.62	20.09
22	$C_{25}H_{19}N_5OS_2$	53.88	5.43	16.74	53.75	5.50	16.68
26	$C_{25}H_{21}N_5OS$	57.34	4.44	20.54	57.62	4.46	20.51
33	$C_{25}H_{20}N_5O_{2.5}S.1/4H_2O$	57.06	6.64	18.15	56.92	6.61	18.06
35	$C_{25}H_{20}N_5O_{2.5}S.3/4H_2O$	56.83	6.95	17.29	56.81	6.62	17.19
37	$C_{25}H_{19}N_5O_2S.1/2H_2O$	58.16	7.12	16.96	57.94	6.91	16.86
39	$C_{40}H_{25}N_5O_2S$	59.54	6.75	13.89	59.51	6.76	13.82
41	$C_{93}H_{47}F_3N_{10}O_4S.1/2CF_3COOH.1/2H_2O$	51.46	5.51	12.63	51.31	5.48	12.50
43	$C_{25}H_{20}N_5O_2S.3HBr.3/2H_2O$	38.49	5.50	13.30	38.06	5.35	12.93
45	$C_{25}H_{21}N_5O_2S$	56.80	6.21	14.05	56.95	6.54	15.42
46	$C_{25}H_{20}N_5O_2S.H_2O$	56.83	6.01	14.73	56.98	5.76	14.84
48	$C_{29}H_{17}N_5O_2SI.2.5H_2O$	44.82	5.02	11.61	44.60	4.54	11.48
52	$C_{25}H_{19}N_5S_2$	51.40	5.75	19.99	51.60	5.83	19.64
53	$C_{25}H_{23}N_5S_2$	57.14	7.20	16.65	57.27	7.15	16.62
54	$C_{25}H_{21}N_5OS$	54.52	6.10	21.20	54.52	6.17	21.18
55	$C_{30}H_{29}N_5OS$	59.99	7.35	17.50	59.89	7.56	17.35
56	$C_{35}H_{27}N_5OS$	57.33	4.44	20.58	57.44	4.48	20.52
57	$C_{35}H_{29}N_5OS$	59.32	5.97	18.65	58.52	5.59	18.12
60b	$C_{11}H_6NO_3SHg$	26.25	2.20	3.40	26.20	2.19	3.46
61a	$C_6H_3O_2SI.0.5H_2O$	26.01	2.18	—	25.74	2.03	—

What is claimed is:

1. A pharmaceutical composition comprising a compound of the formula:



wherein X is O or S, with the proviso that at least one X is S; R is an alkyl of 1–12 carbons which may be substituted with an hydroxy, amino or halo group; and R^1 is a furyl or thienyl group, or a phenyl group substituted with a substituent R^2 which is a member selected from the group consisting of $—CONH(CH_2)_nNHR^3$ where n=2–10, alkyoxyamide, benzyloxyamide, and alkylamino, and R^3 is alkyl, alkylcarbonyl, or alkoxyamidoalkyl, and a pharmaceutically acceptable carrier.

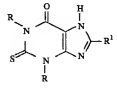
2. The composition of claim 1 which is an elixir.

3. The composition of claim 1 which is in tablet form.

4. The composition of claim 1 adapted for administration through the mucosa.

5. The composition of claim 4 which is adapted for inhalation.

6. A pharmaceutical composition comprising a compound of the formula:



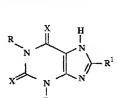
wherein R is an alkyl of 1–12 carbons which may be substituted with an hydroxy, amino or halo group; and R^1 is a cycloalkyl, and a pharmaceutically acceptable carrier.

7. The composition of claim 6, wherein R^1 is cyclopentyl.

8. The composition of claim 7, wherein said compound is 1,3-dipropyl-8-cyclopentyl-2-thioxanthine.

9. The composition of claim 7, wherein said compound is 1,3-dimethyl-8-cyclopentyl-2-thioxanthine.

10. A compound of the formula:



wherein X is O or S, with the proviso that at least one X is S; R an alkyl of 1–12 carbons which may be substituted with an hydroxy, amino or halo group; and R^1 is a furyl or thienyl group, or a phenyl group substituted with a substituent R^2 which is a member selected from the group consisting of $—CONH(CH_2)_nNHR^3$ where n=2–10, alkoxyamide, benzyloxyamide, and alkylamino, and R^3 is alkyl, alkylcarbonyl, or alkoxyamidoalkyl.

11. The composition of claim 1, wherein said compound is 1,3-dimethyl-8-(2-thienyl)-2-thioxanthine.

12. The composition of claim 1, wherein said X at the 2-position is S.

13. The composition of claim 12, wherein R^1 is a phenyl group substituted with a substituent R^2 which is a member selected from the group consisting of alkoxyamide, benzyloxyamide, alkoxyamidoalkyl, and $—CONH(CH_2)_nNHR^2$ where n=2–10, and R^3 is alkyl, alkylcarbonyl, or alkoxyamidoalkyl.

14. The composition of claim 1, wherein said compound is 1,3-dipropyl-8-(2-thienyl)-2-thioxanthine.

15. The composition of claim 10, wherein R^1 is furyl.

16. The composition of claim 10 wherein R^1 is thienyl.

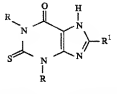
17. The compound of claim 10, wherein said X at the 2-position is S.

18. The compound of claim 17, wherein R^1 is furyl.

19. The compound of claim 17, wherein R^1 is thienyl.

20. The compound of claim 17, wherein R^1 is a phenyl group substituted with a substituent R^2 which is a member selected from the group consisting of $—CONH(CH_2)_nNHR^3$ where n=2–10, alkoxyamide, henzyloxyamide and alkylamino, and R^3 is alkyl, alkylcarbonyl, or alkoxyamidoalkyl.

21. A compound of the formula:



wherein R is an alkyl of 1–12 carbons which may be substituted with an hydroxy, amino or halo group; and R^1 is a cycloalkyl.

22. The compound of claim 21, wherein R^1 is cyclopentyl.

23. The compound of claim 22, wherein said compound is 1,3-dipropyl-8-cyclopentyl-2-thioxanthine.

24. The composition of claim 22, wherein said compound is 1,3-dimethyl-8-cyclopentyl-2-thioxanthine.

25. The compound of claim 10, wherein said compound is 1,3-dimethyl-8-(2-thienyl)-2-thioxanthine.

26. The compound of claim 10, wherein said compound is 1,3-dipropyl-8-(2-thienyl)-2-thioxanthine.

* * * * *

Exhibit S

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Regulation of Ca^{2+}-dependent Desensitization in the Vanilloid Receptor TRPV1 by Calcineurin and cAMP-dependent Protein Kinase*

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The vanilloid receptor TRPV1 is a polymodal nonselective cation channel of nociceptive sensory neurons involved in the perception of inflammatory pain. TRPV1 exhibits desensitization in a Ca^{2+}-dependent manner upon repeated activation by capsaicin or protons. The cAMP-dependent protein kinase (PKA) decreases desensitization of TRPV1 by directly phosphorylating the channel presumably at sites Ser^{116} and Thr^{370}. In the present study we investigated the influence of protein phosphatase 2B (calcineurin) on Ca^{2+}-dependent desensitization of capsaicin- and proton-activated currents. By using site-directed mutagenesis, we generated point mutations at PKA and protein kinase C consensus sites and studied wild type (WT) and mutant channels transiently expressed in HEK293t or HeLa cells under whole cell voltage clamp. We found that intracellular application of the cyclosporin A-cyclophilin A complex (CsA·CyP), a specific inhibitor of calcineurin, significantly decreased desensitization of capsaicin- or proton-activated TRPV1-WT currents. This effect was similar to that obtained by extracellular application of forskolin (FSK), an indirect activator of PKA. Simultaneous applications of CsA·CyP and FSK in varying concentrations suggested that these substances acted independently from each other. In mutation T370A, application of CsA·CyP did not reduce desensitization of capsaicin-activated currents as compared with WT and to mutant channels S116A and T144A. In a double mutation at candidate protein kinase C phosphorylation sites, application of CsA·CyP or FSK decreased desensitization of capsaicin-activated currents similar to WT channels. We conclude that Ca^{2+}-dependent desensitization of TRPV1 might be in part regulated through channel dephosphorylation by calcineurin and channel phosphorylation by PKA possibly involving Thr^{370} as a key amino acid residue.

The capsaicin receptor TRPV1, a nonselective cation channel expressed predominantly in nociceptive sensory neurons, transduces and integrates various stimuli such as noxious heat (>42 °C), capsaicin, protons, (1, 2), the endogenous cannabinoid anandamide (3), lipoxygenase products, and other lipids related to arachidonic acid (4) and ethanol (5). Studies on TRPV1 gene knock-out mice suggest that TRPV1 is essential for the development of thermal hyperalgesia following inflammation or local injection of bradykinin and nerve growth factor (6–8).

Activation of TRPV1 leads to Ca^{2+} influx into nociceptive sensory neurons, resulting in membrane depolarization and release of proinflammatory neuropeptides from primary afferent nerve terminals (9). Prolonged or repeated activation of TRPV1 results in desensitization and insensitivity of the receptor to subsequent stimuli (10, 11). The physiological role and importance of TRPV1 desensitization is unknown but speculated to be a process of adaptation and regulation of the peripheral nervous system for the perception of pain. Comparable with other ion channels, desensitization of TRPV1 is at least in part a Ca^{2+}-dependent process (10, 11). There is growing evidence for the involvement of Ca^{2+}-dependent phosphorylation and dephosphorylation processes to regulate desensitization and excitability of TRPV1. Previous studies in rat dorsal root ganglion neurons have demonstrated that desensitization is reduced in the presence of inhibitors of the Ca^{2+}- and calmodulin-dependent protein phosphatase 2B (calcineurin) (12). Conversely, phosphorylation of TRPV1 by Ca^{2+}-calmodulin-dependent kinase II (CaMKII)[1] seems to be a prerequisite for activation of TRPV1 by capsaicin (13).

Another candidate involved in the mechanisms of Ca^{2+} negative feedback and Ca^{2+}-dependent inactivation in many ion channels is the Ca^{2+} sensor calmodulin (CaM) itself. There is growing evidence that multiple regions of TRPV1 indeed may bind CaM (14, 15).

TRPV1 is also a target for cAMP-dependent protein kinase (PKA)- and protein kinase C (PKC)-dependent phosphorylation. Phosphorylation by PKA sensitizes the channel to heat (16) and capsaicin (17) and reduces Ca^{2+}-dependent desensitization of capsaicin- and proton-activated currents (18, 19). Amino acids residues Ser^{116} and Thr^{370} are the major substrates for PKA-dependent phosphorylation, although other putative PKA phosphorylation sites might be involved as well. Phosphorylation by PKC sensitizes the channel to capsaicin, protons, and heat (20–22). Here, residues Ser^{502} and Ser^{800} are the major substrates for PKC-dependent phosphorylation.

In the present study we investigated the influence of cal-

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[1] The abbreviations used are: CaMKII, Ca^{2+}-calmodulin-dependent kinase II; CaM, calmodulin; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; WT, wild type; CsA, cyclosporin A; CyP, cyclophilin A; CsA·CyP, cyclosporin A-cyclophilin A complex; FSK, forskolin; HEK, human embryonic kidney; PMA, phorbol 12-myristate 13-acetate; OA, okadaic acid; W-7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride.

cineurin on Ca^{2+}-dependent desensitization of capsaicin- and proton-activated currents and examined the interactions of calcineurin and PKA and PKC phosphorylation pathways. We found that Ca^{2+}-dependent desensitization of TRPV1 might be in part regulated through channel dephosphorylation by calcineurin and channel phosphorylation by PKA possibly involving Thr^{370} as a key amino acid residue.

EXPERIMENTAL PROCEDURES

Site-directed Mutagenesis and Transient Transfection—Mutagenesis of rat TRPV1-cDNA was performed with rTRPV1-pcDNA3 by means of the transformer site-directed mutagenesis kit (BD Biosciences Clontech, Palo Alto, CA) as described previously (19). Human embryonic kidney (HEK) 293t cells or cells of a human adenocarcinoma-derived cell line (HeLa cells) were transfected with wild type or mutant plasmid (0.75 or 10 μg, respectively) along with reporter plasmid (CD8-pih3m, 1 μg) by the calcium phosphate precipitation method. After incubation for 12–15 h, the cells were replated in 35-mm culture dishes. Transfected cells were used for experiments within 2–3 days. Transfection-positive cells were identified by immunobeads (anti-CD-8 Dynabeads; Dynal Biotech, Oslo, Norway). Transfection efficiency was ~50–70% on average for TRPV1-WT and mutant channels.

Chemicals and Solutions—Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) and cyclosporin A (CsA; both Sigma-Aldrich) were dissolved in absolute ethanol to give stock solutions of 10 mM. Forskolin (FSK; Calbiochem-Novabiochem GmbH, Bad Soden, Germany), phorbol 12-myristate 13-acetate (PMA; Calbiochem-Novabiochem GmbH), and okadaic acid (OA; Alomone Labs, Ltd., Jerusalem, Israel) were dissolved in dimethyl sulfoxide to give stock solutions of 10, 1, and 1 mM, respectively. Human brain CaM and *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W-7; both Calbiochem-Novabiochem GmbH) were dissolved in double distilled water to give stock solutions of 100 mM. Cyclophilin A (CyP; Sigma-Aldrich) was dissolved in Tris-Cl, pH 7.4, containing HEPES, 1,4-dithio-DL-threitol, phenylmethanesulfonyl fluoride, and sodium azide to give a stock solution of 20 μM. All of the stock solutions were stored at −20 °C. All of the control and test solutions were applied with a polytetrafluorethylen glass multiple-barrel perfusion system. Standard bath solutions contained 70 mM NaCl, 70 mM choline Cl, 5 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES, and 10 mM glucose (adjusted to pH 7.4 with tetramethylammonium hydroxide). The NaCl/Choline Cl composition was used to reduce the amplitude of the Ca^{2+} currents. Choline Cl did not have any influence on WT or mutant channels. Pipette solution contained 140 mM KCl, 2 mM $MgCl_2$, 5 mM EGTA, and 10 mM HEPES (adjusted to pH 7.4 with KOH).

Electrophysiological Technique and Data Analysis—Currents were recorded at room temperature with the whole cell configuration of the patch-clamp method. Holding potential was −60 mV. Patch pipettes were pulled from borosilicate glass tubes (TW150F-3; World Precision Instruments, Sarrasota, FL) and heat-polished at the tip to give a resistance of 0.8–1.2 MΩ. The currents were recorded with an Axopatch 200B patch-clamp amplifier (Axon Instruments, Union City, CA), filtered at 1 kHz, and sampled at 2 kHz. pCLAMP 8.0.1 software (Axon Instruments) was used for acquisition and analysis of currents. Origin 6.1 software (OriginLab Corporation, Northampton, MA) was used to perform least squares fitting and to create figures. The data are presented as the means ± S.E. or fitted value ± S.E. of the fit. An unpaired Student's *t* test (SigmaStat; SSPS Science, Chicago, IL) was used to evaluate the significance of changes in mean values. p values <0.05 were considered statistically significant.

RESULTS

Inhibition of Calcineurin Decreases Ca^{2+}-dependent Desensitization of Capsaicin-activated TRPV1-WT Currents—TRPV1 channel exhibits desensitization in a Ca^{2+}-dependent manner (10, 11). It has been suggested that a rise in cytosolic Ca^{2+} level caused by TRPV1 activation results in the activation of Ca^{2+}/calmodulin-dependent protein phosphatases that mediate channel desensitization (12). To test this hypothesis for TRPV1-WT transiently expressed in HEK293t cells, we first studied the effect of various protein phosphatase inhibitors on Ca^{2+}-dependent desensitization of capsaicin-activated TRPV1-WT currents, specifically on the decreasing current response to successive stimulation (tachyphylaxis). We applied

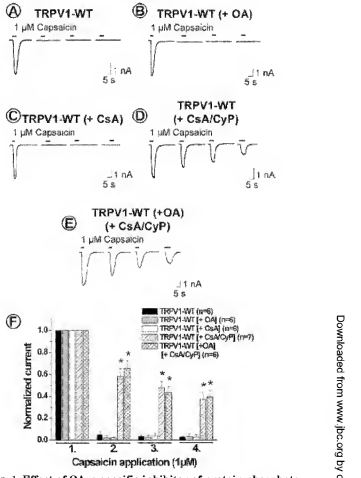

FIG. 1. **Effect of OA, a specific inhibitor of protein phosphatases 1 and 2A, CsA, and CsA·CyP, a specific inhibitor of protein phosphatase 2B (calcineurin), on Ca^{2+}-dependent desensitization of capsaicin-activated TRPV1 currents.** Shown are whole cell current responses of TRPV1 channels to repeated brief (~5 s long) applications of 1 μM capsaicin in Ca^{2+}-containing (2 mM) bath solution without (*A*) or with 1 μM OA (*B*), 100 μM CsA (*C*), or 14 nM CsA + 17 nM CyP in the pipette solution (*D*), or 14 nM CsA + 17 nM CyP in the pipette solution along with 1 μM OA applied extracellularly (*E*). After a whole cell voltage clamp was established, the cells were dialyzed for 10 min before the first capsaicin application. The intervals between capsaicin applications were 2 min. *F*, mean amplitudes of currents ± S.E. measured in experiments as described for *A–E*. The amplitudes were normalized to the current amplitude obtained with first capsaicin application. * indicates a statistically significant difference in the mean amplitude compared with that obtained under control conditions.

a series of brief (~5 s long) pulses of 1 μM capsaicin at 2-min intervals in Ca^{2+}-containing solution (2 mM) and measured the current response. Under control conditions, TRPV1-WT showed strong tachyphylaxis (Fig. 1*A*). Most of the tachyphylaxis occurred between the first and second application, as described previously (19). The mean current amplitudes at the second and fourth capsaicin application were 5.1 ± 2.7 and 3.1 ± 0.4% of that of the first application, respectively (Fig. 1*F*). Pretreatment of cells for 10 min with 1 μM OA in the pipette solution, an inhibitor of protein phosphatase 1 and 2A, did not lead to any change in Ca^{2+}-dependent tachyphylaxis of capsaicin-activated currents (Fig. 1*B*). Here, current amplitudes at the second and fourth capsaicin application were 2.5 ± 1.6 and 3.7 ± 2.4% of that of the first application, respectively (Fig. 1*F*). Similarly, OA was without any effect on Ca^{2+}-dependent tachyphylaxis when used in both lower or higher concentrations (0.01–100 μM, data not shown). In contrast, tachyphylaxis was significantly decreased when cells were pretreated for 10 min

TABLE I

Mean amplitudes of currents (means ± S.E.) evoked by the first capsaicin/proton application in n experiments under various experimental conditions

$p < 0.05$ indicates a statistically significant difference in the mean amplitude compared with that obtained under control conditions.

Channel	Activator	Experimental condition	Response	*n*	*p*
			nA		
TRPV1-WT	Capsaicin		6.5 ± 1.6	6	
TRPV1-WT	Capsaicin	100 μM OA in pipette	9.3 ± 2.6	6	
TRPV1-WT	Capsaicin	100 μM CsA in pipette	6.8 ± 1.2	6	
TRPV1-WT	Capsaicin	14 nM CsA, 17 nM CyP in pipette	4.8 ± 1.2	7	
TRPV1-WT	Capsaicin	100 μM OA plus 14 nM CsA, 17 nM CyP in pipette	4.3 ± 1.2	6	
TRPV1-WT	Capsaicin (30 *s*)		7.8 ± 1.8	6	
TRPV1-WT	Capsaicin (30 *s*)	14 nM CsA, 17 nM CyP in pipette	5.0 ± 1.1	7	
TRPV1-WT	Capsaicin	100 μM W-7 in pipette	7.8 ± 1.7	8	
TRPV1-WT	Capsaicin	100 μM CaM in pipette	8.6 ± 2.0	6	
TRPV1-WT	Capsaicin	10 μM FSK in extracellular buffer	5.2 ± 0.9	9	
TRPV1-S116A	Capsaicin		8.9 ± 2.3	7	
TRPV1-S116A	Capsaicin	14 nM CsA, 17 nM CyP in pipette	6.1 ± 1.2	7	
TRPV1-T370A	Capsaicin		2.3 ± 0.6	6	<0.05
TRPV1-T370A	Capsaicin	14 nM CsA, 17 nM CyP in pipette	3.2 ± 0.8	6	
TRPV1-T144A	Capsaicin		2.8 ± 0.6	6	
TRPV1-T144A	Capsaicin	14 nM CsA, 17 nM CyP in pipette	3.9 ± 1.0	6	
TRPV1-WT	Capsaicin	0.1 μM PMA in extracellular buffer	5.3 ± 2.3	6	
TRPV1-S502A/S800A	Capsaicin		6.2 ± 2.3	6	
TRPV1-S502A/S800A	Capsaicin	14 nM CsA, 17 nM CyP in pipette	4.6 ± 1.2	8	
TRPV1-WT	Proton		3.1 ± 0.6	6	
TRPV1-WT	Proton	14 nM CsA, 17 nM CyP in pipette	4.2 ± 0.7	6	
TRPV1-WT	Proton (30*s*)		6.4 ± 1.5	6	
TRPV1-WT	Proton (30*s*)	14 nM CsA, 17 nM CyP in pipette	4.0 ± 0.8	6	

with the immunosuppressive drug cyclosporin A (CsA; 14 nM) together with the "immunophilin" cyclophilin A (CyP; 17 nM) in the pipette solution (Fig. 1*D*). CsA and CyP are known to form a drug/immunophilin complex, which associates with and thus inhibits protein phosphatase 2B (calcineurin) (23). In the presence of CsA/Cyp, current amplitudes at the second and fourth capsaicin application were 58.4 ± 6.9 and 37.2 ± 6.2% of that at the first application, respectively (Fig. 1*F*). This is in good agreement with previously reported results obtained in rat dorsal root ganglion neurons using similar concentration of CsA and CyP in the pipette solution (12). Higher concentrations of CsA (up to 1 μM) along with CyP (up to 1 μM) did not lead to any further decrease in tachyphylaxis compared with that observed with 14 nM CsA plus 17 nM CyP (data not shown). Pretreatment of cells with CsA alone up to concentrations of 100 μM in the pipette solution did not lead to any change in Ca^{2+}-dependent tachyphylaxis of capsaicin-activated currents compared with control conditions, indicating that indeed CsA complexed to CyP is the active form that specifically targets calcineurin (Fig. 1, *C* and *F*). Pretreatment of cells for 10 min with 14 nM CsA and 17 nM CyP along with 1 μM OA in the pipette solution did not lead to any further decrease in tachyphylaxis compared with that observed with CsA·CyP (Fig. 1, *E* and *F*). Pretreatment of cells with OA, CsA, or CsA·CyP in the pipette solutions did not have any significant effect on capsaicin-activated peak currents of TRPV1-WT (Table I).

We also investigated responses of TRPV1-WT channels to prolonged applications (30-s) of 1 μM capsaicin in Ca^{2+}-containing (2 mM) bath solution. TRPV1-WT currents had peak amplitudes in the range of 2.6–14.4 nA with a mean of 7.1 ± 1.8 nA. Currents reached their peak at 1.6 ± 0.5 s after beginning of activation. The currents then began to decrease rapidly during continuous capsaicin application and reached values of 0.41 ± 0.18, 0.34 ± 0.06, and 0.21 ± 0.03 nA after 10, 20, and 30 s, respectively (Fig. 2*A*). This type of desensitization has been termed acute desensitization, consistent with previous reports (10, 11, 19).

Pretreatment of cells for 10 min with 14 nM CsA plus 17 nM CyP in the pipette solution also led to a significant decrease in acute desensitization of capsaicin-activated TRPV1-WT currents. The currents had peak amplitudes in the range of 2.4-8.9 nA with a mean of 5.0 ± 1.1 nA, which was not significantly different from control conditions (Table I). The currents reached their peak at 1.9 ± 0.7 s after beginning of activation. Then the currents began to decrease during continuous capsaicin application and reached values of 2.18 ± 0.62, 0.64 ± 0.08, and 0.59 ± 0.05 nA after 10, 20, and 30 s, respectively (Fig. 2*B*). To describe and compare acute desensitization quantitatively for control conditions and in the presence of CsA·CyP, we measured the areas under the current curves over a time of 30 s and normalized them to an idealized (rectangular), nondesensitizing current of a respective size. The data are given in Fig. 2*C*.

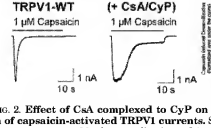

FIG. 2. **Effect of CsA complexed to CyP on acute desensitization of capsaicin-activated TRPV1 currents.** Shown are whole cell current responses to 30-*s*-long applications of 1 μM capsaicin in Ca^{2+}-containing (2 mM) bath solution without (*A*) or with 14 nM CsA + 17 nM CyP in the pipette solution (*B*). After a whole cell voltage clamp was established, the cells were dialyzed for 10 min before the first capsaicin application. *C*, the areas under the current curves were measured and normalized to an idealized, nondesensitizing current of respective size. The *bars* represent mean values ± S.E. * indicates a statistically significant difference compared with control.

Calmodulin Is Not Required for the Decrease in Ca^{2+}-dependent Desensitization by Inhibition of Calcineurin—Calcineurin exerts its phosphatase activity in a Ca^{2+}- and calmodulin-dependent manner. CaM itself is a dominant Ca^{2+} sensor for Ca^{2+}-dependent inactivation in many ion channels (24–26). There is accumulating evidence that multiple regions of TRPV1 may bind CaM. One putative region was identified in the C-terminal (14), and another was identified in the N-terminal segment (15). We investigated the functional role of CaM in Ca^{2+}-dependent desensitization of capsaicin-activated

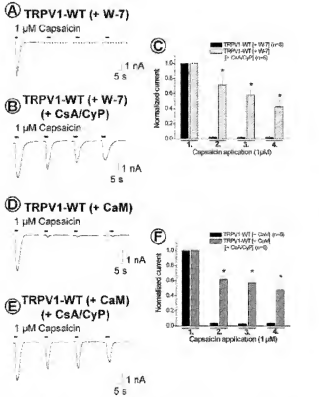

FIG. 3. **Effect W-7, a calmodulin antagonist, and CaM on Ca^{2+}-dependent desensitization of capsaicin-activated TRPV1 currents.** Shown are whole cell current responses of TRPV1 channels to repeated brief (~5-s-long) applications of 1 μM capsaicin in Ca^{2+}-containing (2 mM) bath solution with 100 μM W-7 (*A*) 100 μM W-7 plus 14 nM CsA/17 nM CyP (*B*) 100 μM CaM (*D*) or 100 μM CaM plus 14 nM CsA/17 nM CyP in the pipette solution (*E*). After a whole cell voltage clamp was established, the cells were dialyzed for 10 min before the first capsaicin application. The intervals between capsaicin applications were 2 min. *C* and *F*, The mean amplitudes of currents ± S.E. measured in experiments as described for *A* and *B* or for *D* and *E*, respectively. The amplitudes were normalized to the current amplitude obtained with the first capsaicin application. * indicates a statistically significant difference in the mean amplitudes.

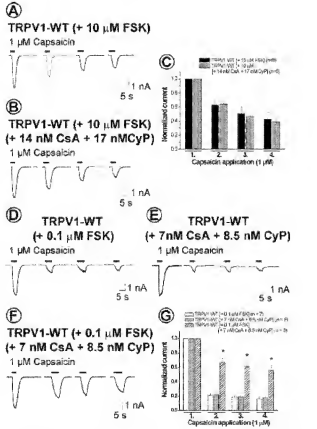

FIG. 4. **Effect of FSK, an indirect activator of protein kinase A, in the absence or presence of CsA complexed to CyP on Ca^{2+}-dependent desensitization of capsaicin-activated TRPV1 currents.** Shown are whole cell current responses of TRPV1-WT channels to repeated brief (~5-s-long) applications of 1 μM capsaicin in Ca^{2+}-containing bath solution after pretreatment for 10 min with 10 μM FSK extracellularly (*A*), 10 μM FSK and the addition of 14 nM CsA + 17 nM CyP to the pipette solution (*B*), 0.1 μM FSK extracellularly (*D*), 7 nM CsA + 8.5 nM CyP in the pipette solution (*E*), or 0.1 μM FSK and the addition of 7 nM CsA + 8.5 nM CyP to the pipette solution (*F*). The intervals between capsaicin applications were 2 min. *C* and *G*, mean amplitudes of currents ± S.E. measured in experiments as described for *A* and *B* and for *D–F*. The amplitudes were normalized to the current amplitude obtained with the first capsaicin application.

TRPV1-WT channels. The experiments were performed as described for those shown in Fig. 1. Pretreatment of cells with 100 μM W-7 in the pipette solution, which is a potent noncompetitive antagonist of calmodulin, did not lead to any change in Ca^{2+}-dependent tachyphylaxis of capsaicin-activated TRPV1-WT currents (Fig. 3, *A* and *C*). W-7 also did not have any significant effect on capsaicin-activated peak currents of TRPV1-WT (Table I). This is in good agreement with an earlier report about the ineffectiveness of W-7 on TRPV1 channel desensitization (14). Tachyphylaxis was significantly decreased when cells were pretreated with 100 μM W-7 along with 14 nM CsA plus 17 nM CyP in the pipette solution (Fig. 3*B*). Here, current amplitudes at the second and fourth capsaicin application were 71.7 ± 12.0 and 42.2 ± 8.1% of that of the first application, respectively (Fig. 3*C*). The decrease in channel tachyphylaxis was comparable with that observed in the presence of CsA plus CyP in the pipette solution (Figs. 1*F* and 3*C*). This indicates that inhibition of calcineurin alone is sufficient to inhibit TRPV1-WT channel desensitization. CaM alone up to concentrations of 100 μM in the pipette solution did not lead to any change in tachyphylaxis of capsaicin-activated TRPV1-WT currents compared with control conditions (Fig. 3, *D* and *F*). Pretreatment of cells with CaM up to concentrations of 100 μM along with 14 nM CsA and 17 nM CyP in the pipette solution led to a significant decrease in tachyphylaxis of capsaicin-activated TRPV1-WT currents (Fig. 3*E*). Here, the results were quantitatively similar to those obtained with CsA·CyP alone or CsA·CyP along with W-7 in the pipette solution (Figs. 1*F* and 3, *C* and *F*).

Phosphorylation by PKA and Dephosphorylation by Calcineurin Regulates Ca^{2+}-dependent Desensitization of TRPV1—In the resting state, TRPV1 is highly phosphorylated, at least when heterologously expressed in CHO-K1 cells (18). PKA is able to phosphorylate TRPV1. However, PKA phosphorylation only becomes obvious in the desensitized state (18). Phosphorylation by PKA partly rescues TRPV1 from desensitization (18, 19). Because inhibition of calcineurin decreases desensitization of capsaicin-activated TRPV1 currents to a similar extent like activation of PKA, we investigated the interplay between PKA activation and calcineurin inhibition and their effect on Ca^{2+}-dependent desensitization of capsaicin-activated TRPV1 currents. As demonstrated previously, pretreatment of cells for 10 min with 10 μM FSK, an activator of adenylate cyclase and thus an indirect PKA activator, led to a significant decrease in channel tachyphylaxis (Fig. 4*A*) (19). Current amplitudes at the second and fourth capsaicin application were 62.7 ± 7.8 and 42.8 ± 9.8% of that at the first application, respectively (Fig. 4*C*). FSK pretreatment did not have any

TABLE II

Effect of various concentrations of FSK and CsA-CyP, applied alone or in combination, on Ca^{2+}-dependent desensitization of capsaicin-activated TRPV1 currents

The experiments were performed as described in the legend to Fig. 4.

FSK treatment	CsA-CyP treatment	Second current[a]	Fourth current[a]	*n*
μM	*nM*	%	%	
0	0	5.1 ± 2.7	3.1 ± 0.4	6
0.1	0	21.5 ± 1.6	16.2 ± 2.1	7
0	1.4/1.7	2.4 ± 0.6	1.1 ± 0.3	5
0.1	1.4/1.7	18.3 ± 1.7	15.7 ± 2.2	3
0.1	0	21.5 ± 1.6	16.2 ± 2.1	7
0	7/8.5	21.9 ± 2.6	17.5 ± 2.4	8
0.1	7/8.5	67.3 ± 5.9	56.0 ± 6.8	8
10	0	62.7 ± 7.8	42.8 ± 9.8	9
0	14/17	58.4 ± 6.9	37.2 ± 6.2	7
10	14/17	64.0 ± 6.8	38.9 ± 6.1	6

[a] The values given for the second and fourth currents are percentages of the values for the first current.

significant effect on capsaicin-activated peak currents in TRPV1-WT (Table I). Pretreatment of cells for 10 min with 14 nM CsA plus 17 nM CyP in the pipette solution along with 10 μM FSK in the external solution did not lead to any significant further decrease in tachyphylaxis compared with that in the presence of 10 μM FSK alone (Fig. 4, *B* and *C*). To exclude the possibility that the decrease in desensitization in the presence of CsA-CyP is caused by PKA phosphorylation caused by disinhibition of PKA by CsA-CyP, we investigated the effect of CsA-CyP on channel tachyphylaxis in the presence of the PKA inhibitor KT5720. In these experiments the decrease in desensitization was similar to that obtained with CsA-CyP alone (data not shown).

To address the question of whether the regulation of desensitization by FSK and CsA-CyP are of an additive or synergistic nature, we also measured the effect of submaximal concentrations of FSK and CsA-CyP on channel tachyphylaxis (Table II). Pretreatment of cells for 10 min with 1.4 nM CsA plus 1.7 nM CyP in the pipette solution did not lead to any significant decrease in tachyphylaxis. With 7 nM CsA plus 8.5 nM CyP in the pipette solution, current amplitudes at the second and fourth capsaicin application were 21.9 ± 2.6 and 17.5 ± 2.4% of that at the first application, respectively (Fig. 4*E*). Comparable effects were obtained when cells were pretreated with 0.1 μM FSK alone (21.5 ± 1.6 and 16.2 ± 2.1%; Fig. 4*D*) or 0.1 μM FSK along with 1.7 nM CsA plus 1.7 nM CyP in the pipette solution (18.3 ± 1.7 and 15.7 ± 2.2%). Pretreatment of cells with 0.1 μM FSK along with 7 nM CsA plus 8.5 nM CyP in the pipette solution, however, led to an effect similar to that obtained with 10 μM FSK along with 14 nM CsA plus 17 nM CyP in the pipette solution (Fig. 4*F*).

These results demonstrate that the concentration dependence for the CsA-CyP-mediated decrease in desensitization is rather steep, as expected for effects that require a cascade of reactions rather than simple one-to-one reactions. Thus, we refrained from performing a more detailed quantitative assessment for the interactions of CsA-CyP with FSK. However, the results obtained by simultaneous application of FSK and CsA-CyP exclude a subadditive action on channel desensitization and are in favor of an additive action for two reasons. First, the effect on tachyphylaxis caused by a submaximal concentration of FSK was unaltered by application of CsA-CyP in a concentration that alone was too weak to effect desensitization. Second, simultaneous application of CsA-CyP and FSK both in concentrations that alone caused a significant but submaximal decrease in channel tachyphylaxis led to a maximal decrease in tachyphylaxis, meaning a decrease that could not be further enhanced by higher concentrations of CsA-CyP or FSK.

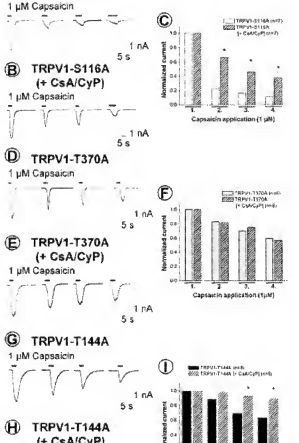

FIG. 5. **Effect of CsA complexed to CyP on Ca^{2+}-dependent desensitization in TRPV1 mutant channels containing the amino acid alanine at putative PKA phosphorylation sites.** Shown are whole cell current responses of mutations S116A (*A* and *B*), T370A (*D* and *E*), and T144A (*G* and *H*) to repeated brief (~5-s-long) applications of 1 μM capsaicin in Ca^{2+}-containing bath solution without (*A*, *D*, and *G*), or with 14 nM CsA + 17 nM CyP in the pipette solution (*B*, *E*, and *H*). After a whole cell voltage clamp was established, the cells were dialyzed for 10 min before the first capsaicin application. The intervals between capsaicin applications were 2 min. *C*, *F*, and *I*, mean amplitudes of currents ± S.E. measured in experiments as described for *A*, *B*, *D*, *E*, *G*, and *H*. The amplitudes were normalized to the current amplitude obtained with the first capsaicin application. * indicates a statistically significant difference between the mean values obtained without and with CsA-CyP treatment.

Among several putative PKA phosphorylation sites of TRPV1, amino acid residues Ser^{116} and Thr^{370} seem to be the most critical ones for PKA-dependent modulation of TRPV1 (18, 19). Substitution of Ser^{116} or Thr^{370} with either alanine or aspartate led to mutant channels that could not be modulated by PKA (19). We now investigated the effect of calcineurin inhibition on Ca^{2+}-dependent desensitization in TRPV1 mutant channels S116A and T370A. Experiments were performed as described for those shown in Fig. 1. Under control conditions, TRPV1-S116A clearly showed some tachyphylaxis that was however less pronounced compared with TRPV1-WT (Fig. 5*A*). Here, the current amplitudes at the second and fourth capsaicin application were 22.6 ± 8.7 and 11.0 ± 4.0% of that of the first application, respectively, and were significantly larger than those for TRPV1-WT under

control conditions (Figs. 1*F* and 5*C*). Pretreatment of TRPV1-S116A for 10 min with 14 nM CsA and 17 nM CyP in the pipette solution, however, led to a significant decrease in tachyphylaxis compared with control conditions (Fig. 5*B*). Current amplitudes at the second and fourth capsaicin application were 66.1 ± 4.1 and 38.2 ± 7.9% of that of the first application, respectively (Fig. 5*C*). This indicates that dephosphorylation by calcineurin of a residue other than Ser^{116} might significantly contribute to desensitization.

As described before (19), TRPV1-T370A is one of the least desensitizing alanine mutation at putative PKA phosphorylation sites under control conditions (Fig. 5*D*). Here, current amplitudes at the second and fourth capsaicin application were 83.2 ± 5.4 and 59.5 ± 7.9% of that of the first application (Figs. 1*F* and 5*F*). Pretreatment for 10 min with 14 nM CsA and 17 nM CyP in the pipette solution, however, had no statistically significant effect on channel tachyphylaxis (Fig. 5*E*). Here, current amplitudes at the second and fourth capsaicin application were 82.1 ± 3.1 and 56.7 ± 5.5% of that of the first application, respectively (Fig. 5*F*). Because mutation T370A constitutively shows only weak tachyphylaxis, it could be speculated that inhibition of calcineurin will not produce any measurable effect on tachyphylaxis, even if the channel was a substrate for calcineurin. To exclude this possibility, we investigated the effect of calcineurin inhibition on mutation T144A, which is another channel exhibiting impaired tachyphylaxis (19). In this mutation, pretreatment for 10 min with 14 nM CsA and 17 nM CyP in the pipette solution significantly decreased tachyphylaxis compared with control conditions (Fig. 5, *G–I*). Pretreatment of cells with CsA-CyP did not have any significant effect on the peak amplitudes of capsaicin-activated currents in any of these mutant channels (Table 1). These observations support the idea that amino acid residue Thr^{370} might be a key site for calcineurin-induced dephosphorylation of TRPV1.

Phosphorylation by PKCε Does Not Modulate Ca^{2+}-dependent Desensitization of Capsaicin-activated TRPV1 Currents—In addition to PKA, the ε-isoform of PKC was demonstrated to directly modify TRPV1 and to sensitize heat- and capsaicin-activated currents (20–22). Here, residues Ser^{502} and Ser^{800} were suggested to be the major substrates for PKC-dependent phosphorylation (22). However, there is no evidence for a PKC-dependent modulation of TRPV1 desensitization so far (19). We were interested in whether or not there is any interplay between PKC phosphorylation and calcineurin-modulation of TRPV1. Experiments were performed as described for those shown in Fig. 1.

As demonstrated before, pretreatment of cells with PMA (0.1 μM), an activator of PKC, did not have any effect on channel tachyphylaxis (Fig. 6*A*). PMA pretreatment also did not have any significant effect on the peak amplitudes of capsaicin-activated currents (Table I). Pretreatment for 10 min with 14 nM CsA and 17 nM CyP in the pipette solution along with 0.1 μM PMA in external buffer significantly decreased channel tachyphylaxis (Fig. 6, *B* and *C*).

Calcineurin inhibition by CsA-CyP also significantly decreased tachyphylaxis in double mutation S502A/S800A, in which putative PKC phosphorylation sites were disrupted (Fig. 6, *D*, *E*, and *G*). In the same double mutation S502A/S800A, FSK decreased tachyphylaxis to a similar extent like CsA-CyP (Fig. 6, *F* and *G*). Pretreatment of cells with CsA-CyP or FSK did not have any significant effect on peak amplitudes of capsaicin-activated currents in mutation S502A/S800A (Table I). These results confirm that phosphorylation of TRPV1 by PKCε indeed is not involved in the channel desensitization process.

Activation of PKA Increases Capsaicin Sensitivity of TRPV1—To determine the effect of calcineurin inhibition by

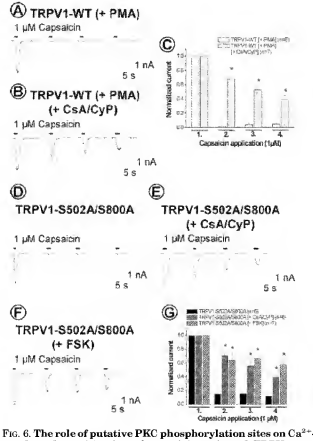

FIG. 6. **The role of putative PKC phosphorylation sites on Ca^{2+}-dependent desensitization of capsaicin-activated TRPV1 currents.** *A* and *B*, whole cell current responses of TRPV1-WT channels to repeated brief (~5 *s long*) applications of 1 μM capsaicin in Ca^{2+}-containing bath solution after pretreatment for 10 min with 0.1 μM PMA extracellularly (*A*) or 0.1 μM PMA and the addition of 14 nM CsA + 17 nM CyP to the pipette solution (*B*). The intervals between capsaicin applications were 2 min. *C*, mean amplitudes of currents ± S.E. measured in experiments as described for *A* and *B*. The amplitudes were normalized to the current amplitude obtained with the first capsaicin application. * indicates a statistically significant difference between the mean values. *D–F*, whole cell current responses of the double mutation TRPV1-S502A/S800A to repeated brief (~5 *s long*) applications of 1 μM capsaicin in Ca^{2+}-containing bath solution without (*D*) or with 14 nM CsA + 17 nM CyP in the pipette solution (*E*) or after pretreatment for 10 min with of 10 μM FSK extracellularly (*F*). The intervals between capsaicin applications were 2 min. *G*, mean amplitudes of currents ± S.E. measured in experiments as described for *D–F*. The amplitudes were normalized to the current amplitude obtained with the first capsaicin application. * indicates a statistically significant difference between the mean values compared with control.

CsA-CyP and PKA activation by FSK on the sensitivity of TRPV1-WT, TRPV1-S116A, and TRPV1-T370A toward capsaicin, we measured the concentration dependence of capsaicin responses in Ca^{2+}-free bath solutions and determined the half-maximal activating concentrations (EC_{50}) before and after pretreatment with 10 μM FSK in the external solution or 14 nM CsA with 17 nM CyP in the pipette solution. Under control conditions, EC_{50} for TRPV1-WT was 242 ± 2 nM, the Hill coefficient (*h*) was 1.9 ± 0.1 (Fig. 7*A*). These values are in reasonable agreement with values found previously for TRPV1 expressed in HEK293t cells (19). The capsaicin concentration-response curve was significantly shifted leftward by ~4.5-fold after pretreatment with FSK (EC_{50} = 52 ± 2 nM; h = 1.8 ± 0.1; Fig. 7*A*) but was not significantly changed after pretreatment with CsA-CyP (EC_{50} = 190 ± 11 nM; h = 1.8 ± 0.2; Fig. 7*A*).

These results confirm earlier reports that the PKA pathway not only regulates desensitization of TRPV1 but also sensitizes

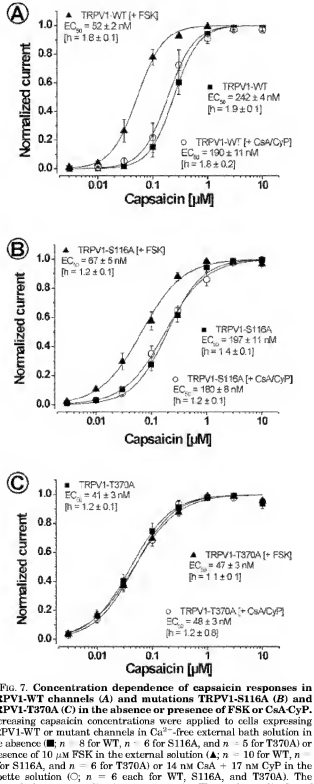

FIG. 7. **Concentration dependence of capsaicin responses in TRPV1-WT channels (*A*) and mutations TRPV1-S116A (*B*) and TRPV1-T370A (*C*) in the absence or presence of FSK or CsA-CyP.** Increasing capsaicin concentrations were applied to cells expressing TRPV1-WT or mutant channels in Ca^{2+}-free external bath solution in the absence (■; $n = 8$ for WT, $n = 6$ for S116A, and $n = 5$ for T370A) or presence of 10 μM FSK in the external solution (▲; $n = 10$ for WT, $n = 6$ for S116A, and $n = 6$ for T370A) or 14 nM CsA + 17 nM CyP in the pipette solution (○; $n = 6$ each for WT, S116A, and T370A). The intervals between applications were 1 min. The peak amplitudes of capsaicin-activated currents were measured, normalized to the maximum response measured in each cell, and plotted against the capsaicin concentration. The *lines* represent the fits of the data to the Hill equation. EC_{50} values and Hill coefficients (h) are given in the figure.

the channel to capsaicin. These results also imply that under resting conditions, the channel is only partly phosphorylated at PKA sites, unlike suggested earlier (18).

For TRPV1-S116A, the EC_{50} was 197 ± 11 nM under control conditions, and the Hill coefficient (h) was 1.4 ± 0.1 (Fig. 7*B*). These values are in reasonable agreement with values found previously for TRPV1-S116A (19). Comparable with WT, in this mutation the capsaicin concentration-response curve was significantly shifted leftward by ~3-fold after pretreatment with FSK (EC_{50} = 67 ± 5 nM; h = 1.2 ± 0.1; Fig. 7*B*) but was not significantly changed after pretreatment with CsA·CyP (EC_{50} = 180 ± 8 nM; h = 1.2 ± 0.1; Fig. 7*B*). Earlier, we have demonstrated that in mutation TRPV1-S116, there was a slight reduction in tachyphylaxis after pretreatment with FSK, which, however, was not statically significant (19). The data in this study confirm that one or several other residues in addition to Ser^{116} are very likely involved in the mechanism of PKA-dependent modulation of TRPV1.

For TRPV1-T370A, sensitivity toward capsaicin was significantly lower under control conditions compared with WT and TRPV1-S116A (EC_{50} = 41 ± 3 nM; h = 1.2 ± 0.1; Fig. 7*C*). In this mutation, pretreatment with FSK or CsA·CyP did not have any significant effect on the EC_{50} values or Hill coefficients. These observations support the idea that amino acid residue Thr^{370} might be a key site for PKA-mediated phosphorylation of TRPV1. Concentration-effect experiments were performed in Ca^{2+}-free solutions to prevent channel desensitization. This way, the ineffectiveness of calcineurin in these experiments can be explained.

Inhibition of Calcineurin Decreases Ca^{2+}-dependent Desensitization of Proton-activated TRPV1-WT Currents—TRPV1 is a multimodal sensor that in addition to vanilloids is also activated by protons and heat (1, 2), anandamide (3), ethanol (5), various lipoxygenase products, and other lipids related to arachidonic acid (4). Although not unequivocal, activation of TRPV1 by protons has been demonstrated to lead to channel desensitization in a Ca^{2+}-dependent manner (11). This desensitization can be partly rescued by PKA activation as well (18).

We studied the effect of calcineurin inhibition on desensitization of proton-activated TRPV1 currents. As HEK293t cells were shown to endogenously express an acid sensing ion channel (hASIC1a) (27), channels were transiently expressed in HeLa cells for these experiments.

Under control conditions, TRPV1-WT showed pronounced proton-induced tachyphylaxis (Fig. 8, *A* and *C*) that was qualitatively similar to capsaicin-induced tachyphylaxis. Proton-induced tachyphylaxis was significantly decreased when cells were pretreated for 10 min with 14 nM CsA plus 17 nM CyP in the pipette solution (Fig. 8, *B* and *C*). Similarly, CsA·CyP decreased acute desensitization of TRPV1-WT induced by prolonged application (30 s) of protons (Fig. 8, *D–F*). Peak amplitude of proton-activated currents were unaffected by CsA·CyP (Table I).

DISCUSSION

In this study, we show that specific inhibition of calcineurin (protein phosphatase 2B) significantly decreases Ca^{2+}-dependent desensitization of capsaicin- and proton-activated TRPV1 currents. This effect is qualitatively and quantitatively similar to but independent from that obtained by extracellular application of FSK and cannot be further enhanced by simultaneous application of FSK and CsA·CyP. In mutation T370A, but not mutations S116A and T144A, desensitization properties are unaffected by calcineurin inhibition. In double mutation S502A/S800A, in which putative PKC phosphorylation sites are disrupted, both calcineurin inhibition and PKA activation decrease desensitization of capsaicin-activated currents similar to WT channels. We conclude that Ca^{2+}-dependent desensitization of TRPV1 might be in part regulated through channel dephosphorylation by calcineurin and channel phosphorylation by PKA, possibly involving Thr^{370} as a key amino acid residue.

Dephosphorylation and Desensitization—Protein phosphorylation and dephosphorylation is a major mechanism in mam-

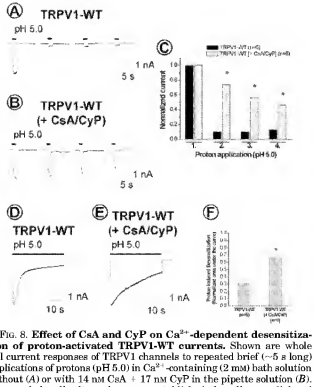

FIG. 8. **Effect of CsA and CyP on Ca^{2+}-dependent desensitization of proton-activated TRPV1-WT currents.** Shown are whole cell current responses of TRPV1 channels to repeated brief (~5 s long) applications of protons (pH 5.0) in Ca^{2+}-containing (2 mM) bath solution without (*A*) or with 14 nM CsA + 17 nM CyP in the pipette solution (*B*). After a whole cell voltage clamp was established, the cells were dialyzed for 10 min before the first capsaicin application. The intervals between proton applications were 2 min. *C*, mean amplitudes of currents + S.E. measured in experiments as described for *A* and *B*. The amplitudes were normalized to the current amplitude obtained with the first proton application. * indicates a statistically significant difference in the mean amplitude compared with control. *D* and *E*, whole cell current responses of TRPV1 channels evoked by a 30-s-long application of protons (pH 5.0) in Ca^{2+}-containing (2 mM) bath solution without (*D*) and with 14 nM CsA + 17 nM CyP in the pipette solution (*E*). *F*, the areas under the current curves were measured and normalized to an idealized, nondesensitizing current of respective size. The *bars* represent the mean values + S.E. * indicates a statistically significant difference compared with control.

malian cells for regulating structure and function and responding to external stimuli. In a previous study, ^{32}P labeling and immunoprecipitation revealed that TRPV1 is highly phosphorylated in the resting state, at least when heterologously expressed in CHO-K1 cells, and that phosphorylation could be significantly reduced by application of a desensitizing concentration of capsaicin (18). In another study, dephosphorylation could be prevented by coapplication of capsaicin and FK-506, another calcineurin inhibitor. Moreover, evidence was presented that phosphorylation of TRPV1, presumably at a CaMKII consensus phosphorylation site (Ser^{502}/Thr^{704}), is a prerequisite for the capsaicin binding capacity of TRPV1 (13).

The decrease in desensitization by the CsA·CyP complex found in this study confirms previously reported results obtained in rat dorsal root ganglion neurons (12) and strongly suggests that dephosphorylation by calcineurin indeed comprises channel activity and thus elicits desensitization. Because calcineurin is a Ca^{2+}- and calmodulin-dependent phosphatase, this mechanism could account for the Ca^{2+} dependence of desensitization. Similar mechanisms have been suggested to underlie the desensitization of the ionotropic receptor P2X3 (28), the neuronal nicotinic acetylcholine receptor (29) and the *N*-methyl-D-aspartate receptor (30, 31). Interestingly, CsA alone had no effect on desensitization, indicating that HEK293t cells might not contain sufficient cyclophilin A to allow formation of the inhibitory complex, at least not under our experimental conditions.

Calcineurin inhibition not only decreased channel tachyphylaxis but also decreased acute desensitization elicited by a 30-s-long capsaicin application, an effect that could not be observed with PKA activation in an earlier study (19). This disparity in effects between calcineurin inhibition and PKA activation suggests that dephosphorylation might be a faster process as compared with rephosphorylation. Moreover, dephosphorylation might not require a closed or ligand-free channel, as hypothesized for the process of rephosphorylation.

Calcineurin inhibition also decreased desensitization of proton-activated TRPV1 currents under our experimental conditions. Unlike suggested by others (13), we conclude that activation by capsaicin and activation by protons most probably initiate comparable mechanisms of Ca^{2+}-dependent desensitization.

The Role of CaM—Calcineurin acts in a Ca^{2+}- and calmodulin-dependent manner. CaM is a dominant Ca^{2+} sensor for Ca^{2+}-dependent inactivation in many ion channels (24–26). There is accumulating evidence that multiple regions of TRPV1 may bind CaM. One putative region was identified in the C-terminal (14), and another was identified in the N-terminal segment (15).

In our experiments both CaM and the calmodulin antagonist W-7 did not have any effect on TRPV1 desensitization or peak amplitudes of capsaicin-activated currents. The ineffectiveness of the CaM inhibitor is in good agreement with an earlier report (14) and might suggest that CaM is not involved in Ca^{2+}-dependent desensitization of capsaicin-activated TRPV1 currents. However, CaM inhibitors would only be expected to inhibit desensitization if CaM acted as a free molecule (32, 33). This, however, might not be the case as suggested by recent reports (15, 22).

CaM was demonstrated to mediate Ca^{2+} inhibition of TRPV1 in inside-out excised patches of *Xenopus* oocytes and HEK293 cells expressing TRPV1. In that study, CaM was applied to the intracellular site of the channel together with 50 μM free Ca^{2-} (15). We cannot exclude that the ineffectiveness of CaM in our study is due to the rather uncontrolled intracellular free Ca^{2-} concentration under our experimental conditions. Thus, from our data, we cannot derive profound evidence for or against a role of CaM in TRPV1-WT channel desensitization.

Functional Coupling of Calcineurin and PKA—The decrease in desensitization of capsaicin-activated currents by calcineurin inhibition in this study was qualitatively and quantitatively similar to that obtained by PKA activation. Simultaneous application of FSK and CsA·CyP in submaximal and maximal concentrations suggest additive actions of FSK and CsA·CyP rather than subadditive or synergistic actions. In mutation T370A, in which a putative PKA phosphorylation site is disrupted, desensitization properties were unaffected by calcineurin inhibition. Calcineurin inhibition, however, did reduce desensitization in both mutations S116A, in which another critical PKA phosphorylation site is disrupted (18, 19), and mutation T144A, which shares similar desensitization properties with mutation T370A (19). These results suggest that Ca^{2+}-dependent desensitization of TRPV1 might be in part regulated through channel dephosphorylation by calcineurin and channel phosphorylation by PKA possibly involving Thr^{370} as a key amino acid residue. A functional coupling of calcineurin and PKA was proposed before in mouse ventricular myocytes to control Ca^{2+} influx through Ca^{2+} channels and Ca^{2+} release through ryanodine receptors (34). In these cells, immunofluorescence also revealed colocalization of calcineurin and PKA.

We hypothesize that a similar mechanism could control excitability of nociceptive sensory neurons by regulating desensitization and thus channel availability. The interplay between

CaMKII and PKA remains to be characterized in future studies.

TRPV1 channel phosphorylation by PKA (16) and PKC (22) to control activation thresholds and TRPV1 channel phosphorylation/dephosphorylation by PKA (18, 19), CaMKII (13), and calcineurin to regulate desensitization/availability might allow fine tuning of the nociceptor in response to a noxious environment.

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Competitive Inhibition by Capsazepine of [^{3}H]Resiniferatoxin Binding to Central (Spinal Cord and Dorsal Root Ganglia) and Peripheral (Urinary Bladder and Airways) Vanilloid (Capsaicin) Receptors in the Rat

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ABSTRACT

Capsazepine was reported to block capsaicin- and resiniferatoxin (RTX)-induced responses both *in vivo* and *in vitro* with Schild plots suggesting a competitive mechanism of action. We have used the [^{3}H]RTX binding assay, thought to represent the vanilloid (capsaicin) receptor, to explore the inhibitory mechanism of capsazepine at the receptor level in the rat. In competition assays, capsazepine inhibited [^{3}H]RTX binding by spinal cord, dorsal root ganglion (DRG) and urinary bladder membranes with similar K_i values of 4.0 ± 0.3, 3.5 ± 0.5 and 5.0 ± 1.0 μM (mean ± S.E.M.; three determinations), respectively. By contrast, capsazepine was 35- to 50-fold more potent for inhibiting specific [^{3}H]RTX binding in the airways (K_i = 0.12 ± 0.02 μM; mean ± S.E.M.; four determinations). In experiments in which the concentration of [^{3}H]RTX was varied, 10 μM capsazepine reduced the affinity of the vanilloid receptor expressed by DRG and spinal cord membranes for [^{3}H]RTX from 15 ± 3 to 43 ± 5 pM, and from 20 ± 3 to 80 ± 5 pM (mean ± S.E.M.; three determinations), respectively, without a measurable change in B_{max} or in cooperativity index; these shifts in affinity yield K_i values of 5.2 and 3.3 μM for DRG and spinal cord membranes, respectively. Capsaicin inhibited [^{3}H]RTX binding by spinal cord, DRG and urinary bladder membranes with a 6- to 13-fold higher potency than did capsazepine; the K_i values were 0.3 ± 0.1, 0.6 ± 0.4 and 0.5 ± 0.2 μM (mean ± S.E.M.; three determinations), respectively. By contrast, in the airways capsaicin and capsazepine competed with equal potency for specific [^{3}H]RTX binding sites (K_i values were 0.12 ± 0.02 μM for capsazepine and 0.14 ± 0.10 μM for capsaicin; mean ± S.E.M.; three determinations). Our results confirm the competitive mechanism of action of capsazepine and, in addition, suggest heterogeneity in the structure-activity relations of vanilloid receptors in different tissues of the rat.

Capsaicin, the pungent principle in hot peppers, is unique among the irritant compounds in that, after an initial stimulation, it renders a subset of sensory neurons, known as primary afferent neurons, insensitive to further stimuli (see Buck and Burks, 1986; Holzer, 1991 for reviews). This refractory state is generally termed "desensitization" (Jancso, 1968). Excitation and subsequent desensitization by capsaicin has long been in use as a neuropharmacological tool to identify primary afferent neurons involved in nociception, neurogenic inflammation, thermoregulation and a variety of reflex responses (see Buck and Burks, 1986; Maggi and Meli, 1988; Holzer, 1991 for reviews). Moreover, desensitization by capsaicin or related compounds is a promising therapeutic approach for various disease states (see Carter, 1991; Maggi, 1991; Rumsfield and West, 1991 for reviews). Although commercial capsaicin creams (Zostrix and Axsain) are already available, these preparations appear to act as counterirritants rather than as desensitizing agents (Dubner, 1991), and the therapeutic use of systemic capsaicin desensitization has been prevented until recently by the lack of specific capsaicin antagonists.

A synthetic benzazepine derivative of capsaicin, called capsazepine, has recently been reported to inhibit capsaicin-induced *in vivo* and *in vitro* responses with Schild plots suggestive of a competitive mechanism of action (Dray *et al.*, 1991; Dickenson and Dray, 1991; Urban and Dray, 1991; Bevan *et al.*, 1992). Capsaicin binds to a specific recognition site on primary afferent neurons which also recognizes other irritant compounds including the ultrapotent capsaicin analog RTX (Szallasi and Blumberg, 1990a). This specific binding site, referred to as the vanilloid receptor (Szallasi and Blumberg, 1990b), can be examined by using radiolabeled RTX. Capsazepine was reported to block RTX-induced responses in cultured DRG

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ABBREVIATIONS: RTX, resiniferonol; DRG, dorsal root ganglion; ROPA, resiniferonol 9,12,13-*ortho*-phenylacetate; RR, ruthenium red.

neurons with an affinity of 100 to 200 nM (Bevan *et al.*, 1992); by contrast, capsazepine was found to inhibit [^{3}H]RTX binding by DRG membranes with an unexpectedly poor potency (James *et al.*, 1992). It also turned out that capsazepine has dissimilar potencies for inhibiting capsaicin-induced responses in different preparations, ranging from 10 (isolated guinea pig ileum) to 1000 nM (isolated rat saphenous nerve and hind paw) (Dray *et al.*, 1991). To resolve the diverse potencies of capsazepine, it has been proposed that capsazepine may act on more than one target (Franco-Cereceda and Lundberg, 1992; Satoh *et al.*, 1992; Lou and Lundberg, 1992). Alternatively, the dissimilar potencies of capsazepine in different tissues may be indicative of receptor heterogeneity such as exemplified by antagonists of the tachykinin receptors (Guard and Watson, 1991). To explore this possibility, we have evaluated the binding affinity of capsazepine in a variety of [^{3}H]RTX binding assays representing the cell bodies (DRG), the central endings (spinal cord), as well as peripheral terminals (urinary bladder, airways) of capsaicin-sensitive neurons.

Methods

Preparation of membranes for binding experiments. Binding assays were carried out as described (Szallasi and Blumberg, 1990a, 1992). Briefly, tissues were homogenized with the aid of a Polytron tissue homogenizer in ice-cold buffer A (pH 7.4), which contained (in mM) KCl, 5; NaCl, 5.8; $MgCl_2$, 2; $CaCl_2$, 0.75; glucose, 12; sucrose, 137 and HEPES, 10. Homogenates of urinary bladder and spinal cord were first centrifuged for 10 min at 1000 *g* (4°C), the pellets were discarded and the supernatants were then centrifuged for 30 min at 35,000 *g*. The resulting pellets were resuspended in buffer A and stored at −20°C until assayed. Homogenates of DRG and airways (trachea and the main bronchi) were first filtered over four layers of surgical gauze to remove debris and then centrifuged in a Beckman 12 microfuge at 12,000 *g* for 15 min at 4°C. The pellets were washed once by resuspending in buffer A and centrifuging as described above.

Binding experiments. For saturation binding experiments, aliquots of the particulate fraction protein (spinal cord and DRG, 40–50 μg; urinary bladder and airways, 60–80 μg) in 0.5 ml of buffer A containing 0.25 mg/ml of bovine serum albumin (Cohn fraction V) were incubated in triplicate with increasing concentrations of [^{3}H]RTX (15–500 pM); nonspecific binding was determined in the presence of 100 nM nonradioactive RTX. Binding was also analyzed in the presence of a fixed concentration of [^{3}H]RTX (spinal cord and DRG, 30 pM; urinary bladder, 50 pM; airways, 100 pM) and various concentrations of competing ligands. Binding assay mixtures were usually incubated for 60 min at 37°C; the binding reaction was then terminated by chilling the assay mixture on ice; 100 μg of *alpha*$_1$-acid glycoprotein was added to each tube to reduce nonspecific binding (Szallasi *et al.*, 1992); bound and free [^{3}H]RTX were separated by pelleting the membranes in a Beckman 12 microfuge, and then quantitated by scintillation counting.

Analysis of binding data. Binding data were analyzed by a computer program that calculates specific bound values using measured total and nonspecific binding and the partition coefficient of [^{3}H]RTX between the aqueous phase and the membranes. Binding parameters were determined using the computer program LIGAND (Biosoft, Cambridge, U.K.). Data from saturation binding experiments were also analyzed by the computer program FigP (Biosoft, Cambridge, U.K.) which fits the measured values to the allosteric Hill equation. The concentration of competing ligands which inhibits specific [^{3}H]RTX binding by 50% (IC_{50}) was determined by a computer program fitting a theoretical sigmoidal competition curve to the data; inhibitory constants (K_i) were calculated using the equation of Cheng and Prusoff (1973), $K_i = IC_{50} / (1 + L/K_d)$, where L is the concentration of unbound [^{3}H]RTX in the assay mixture and K_d is the dissociation constant of RTX as determined in the saturation binding experiments. For calculations, percent inhibition values were determined in three independent experiments for each ligand.

Materials. [^{3}H]RTX (37 Ci/mmol) was synthesized by the Chemical Synthesis and Analysis Laboratory, NCI-FCRF (Frederick, MD), whereas nonradioactive RTX was from LC Services (Woburn, MA). Capsazepine was a generous gift of Dr. Iain James (Sandoz Institute for Medical Research, London, U.K.). All the other chemicals used were purchased from Sigma Chemical Co. (St. Louis, MO).

Results

Characterization of specific [^{3}H]RTX binding sites in rat airways. Capsaicin both upon systemic and topical application induces a dramatic inflammatory response in the trachea and bronchi of the rat (Lundberg and Saria, 1983; Didier *et al.*, 1990; Szolcsanyi *et al.*, 1990) suggesting that vanilloid (capsaicin) receptors are present in rat airways. At 37°C, specific binding of [^{3}H]RTX (60 pM) to rat airway membranes attained its peak by 30-min incubation, and then remained at a constant level during the next 2 hr (not shown). Based on these results, a standard incubation period of 60 min was used in the experiments detailed below. Like for DRG and spinal cord membranes (Szallasi and Blumberg, 1993), dissociation from rat airway membranes turned out to have complex kinetics which was dependent both on time and receptor occupancy (not shown). [^{3}H]RTX displayed saturable specific binding to rat airway membranes whereas the nonspecific binding increased linearly with the concentration of radiolabeled RTX (not shown). In accord with the physiological experiments, rat airway membranes bound [^{3}H]RTX with a maximal receptor density of 76 ± 22 fmol/mg of protein (mean ± S.E.M.; three determinations; fig. 1); this density is comparable to the B_{max} value that we reported in rat DRG membranes (78 fmol/mg of protein; Szallasi and Blumberg, 1993) and confirm here (95 fmol/mg of protein, see below). Airway membranes bound [^{3}H]RTX in a noncooperative fashion (fig. 1); this binding behavior agrees with that observed in urinary bladder (Szallasi *et al.*, 1993) but contrasts with the apparent positive cooperativity of binding (compare with fig. 4B) that characterizes both rat DRG and spinal cord membranes (Szallasi and Blumberg, 1993). It

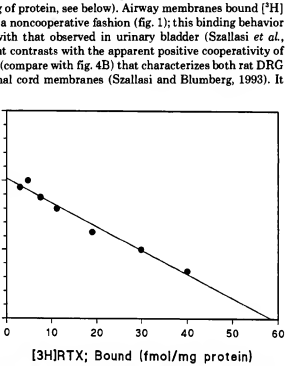

Fig. 1. Scatchard plot of specific [^{3}H]resiniferatoxin binding to rat airway membranes. Points represent mean values from a single experiment; the line was fitted using the LIGAND program. Two additional experiments gave similar results.

should be noted that this positive cooperativity of RTX binding is apparent only at low fractional receptor occupancy, *i.e.*, at low [^{3}H]RTX concentrations (below 20 pM), therefore it could not be detected with the previous binding methodology (Szallasi and Blumberg, 1990a). The curvilinear analysis of the data confirmed the one-site model suggested by the Scatchard plot and gave a final parameter estimate of K_d = 250 ± 26 pM (mean ± S.E.M.; n = 3; fig. 1). This binding affinity is approximately 10-fold lower than that previously determined in DRG, spinal cord and urinary bladder membranes (Szallasi and Blumberg, 1993; Szallasi *et al.*, 1993). At the K_d, nonspecific binding represented approximately 50% of the total binding. Nonradioactive RTX inhibited binding by 50% at the concentration of 350 ± 30 pM (mean ± S.E.M.; three determinations; fig. 2); this value is in good agreement with that determined in saturation binding experiments (K_d = 260 pM; fig. 1B). Specific [^{3}H]RTX binding by rat airway membranes was fully competable by capsaicin (see below), whereas ROPA (1 μM), the C20 deesterified parent compound of RTX which is inactive as a capsaicin analog (Szallasi *et al.*, 1989), did not compete for specific [^{3}H]RTX binding sites (specific binding in the presence of 1 μM ROPA was 108 ± 4% of control binding; mean ± S.E.M.; three determinations; not shown), thereby confirming the specificity of the binding.

Inhibition by capsazepine and capsaicin of specific [^{3}H]RTX binding to rat airway membranes. In the airways, capsaicin and capsazepine competed with equal potency for specific [^{3}H]RTX binding sites: K_i values were 0.14 ± 0.10 μM for capsaicin and 0.12 ± 0.02 μM for capsazepine (mean ± S.E.M.; three determinations; fig. 2). Both capsaicin and capsazepine inhibited [^{3}H]RTX binding to rat airway membranes with approximately 400-fold lower potency than nonradioactive RTX (see above and in fig. 2).

Inhibition by capsazepine and capsaicin of specific [^{3}H]RTX binding to rat spinal cord, DRG and urinary bladder membranes. In competition assays, capsazepine inhibited specific [^{3}H]RTX binding to spinal cord, DRG and urinary bladder membranes with similar K_i values of 4.0 ± 0.3, 3.5 ± 0.5 and 5.0 ± 1.0 μM, respectively (mean ± S.E.M.; three determinations; fig. 3). For spinal cord as well as DRG membranes, K_i values were calculated using the K_d values from the experiments detailed below. For urinary bladder membranes, K_i values were calculated using an affinity (30 pM) determined previously (Szallasi *et al.*, 1993). In experiments in which the concentration of [^{3}H]RTX was varied (15–500 pM), 10 μM capsazepine (the approximate IC_{50} value in competition studies using spinal cord membranes) reduced the affinity of the spinal vanilloid receptor to [^{3}H]RTX from 20 ± 3 to 80 ± 5 pM (mean ± S.E.M.; three determinations) without a measurable change in B_{max} (110 ± 20 fmol/mg of protein in the absence and 135 ± 25 fmol/mg of protein in the presence of 10 μM capsazepine, respectively; mean ± S.E.M.; n = 3) or in cooperativity index (1.7 ± 0.1 and 1.6 ± 0.1 in the absence or presence of capsazepine, respectively; mean ± S.E.M.; three determinations; fig. 4A). These changes in the binding parameters are consistent with the inhibition reflecting a competitive mechanism of action. Moreover, from this shift in affinity, using the equation $K_i = L / (K_{app}/K_d - 1)$, where L is the concentration of capsazepine, and K_{app} and K_d are the concentrations of [^{3}H]RTX at which the half-maximal binding occurs in the presence and absence of capsazepine, respectively, a K_i of 3.3 μM can be calculated. Thus, the affinities of capsazepine to specific [^{3}H]RTX binding sites in the rat spinal cord determined by two distinct protocols (4 and 3.3 μM) are in excellent agreement. In figure 4B, the Scatchard plots of the data from figure 4A are shown. In DRG membranes, as in spinal cord membranes, 10 μM capsazepine reduced the binding affinity from 15 ± 3 to 43 ± 5 pM but had no detectable effect either on maximal binding or on cooperativity index (B_{max} and cooperativity index were 95 ± 10 fmol/mg of protein and 1.8 ± 0.1 in the absence, and 101 ± 5 fmol/mg of protein and 1.6 ± 0.2 in the presence of capsazepine; mean ± S.E.M.; three determinations; not shown). From the shift in affinity using the above equation a K_i of 5.2 μM was calculated. In parallel experiments, capsaicin inhibited specific [^{3}H]RTX binding by spinal cord, DRG and urinary bladder membranes with 6- to 13-fold higher potency than did

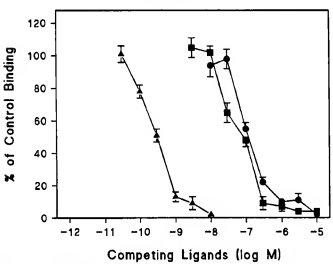

Fig. 2. Inhibition by nonradioactive resiniferatoxin (▲), capsaicin (●) and capsazepine (■) of specific [^{3}H]resiniferatoxin binding to rat airway membranes. Points represent mean values from three independent determinations; error bars indicate ± S.E.M.

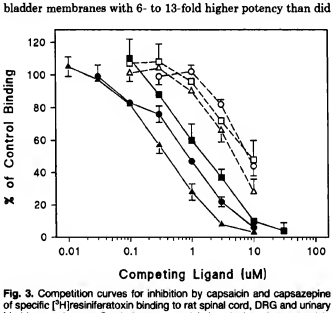

Fig. 3. Competition curves for inhibition by capsaicin and capsazepine of specific [^{3}H]resiniferatoxin binding to rat spinal cord, DRG and urinary bladder membranes. Symbols are: capsaicin in spinal cord, ▲; capsaicin in DRG, ■; capsaicin in bladder, ●; capsazepine in spinal cord, △; capsazepine in DRG, □; capsazepine in bladder, ○. Points represent mean values obtained from three independent determinations; error bars indicate S.E.M.

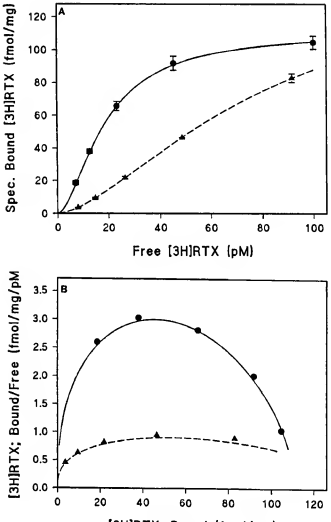

Fig. 4. Specific binding of [^{3}H]resiniferatoxin by rat spinal cord membranes in the absence (●) or presence (▲) of 10 μM capsazepine. A, binding curves; the lines were fitted using the allosteric Hill equation. B, Scatchard plots of the data from A; theoretical curves were generated using the apparent K_d, cooperativity index and B_{max} values from the fit to the allosteric Hill equation. Data are from a single experiment; two additional determinations gave similar results.

capsazepine (fig. 3); K_i values were 0.3 ± 0.1, 0.6 ± 0.4, and 0.5 ± 0.2 μM for spinal cord, DRG and bladder membranes, respectively (mean ± S.E.M.; three determinations).

Discussion

Vanilloid compounds of the capsaicinoid and resiniferanoid classes are thought to share a common binding site, referred to as the vanilloid receptor (Szallasi and Blumberg, 1990b), expressed by a well-defined subpopulation of primary afferent neurons. Upon binding to the vanilloid receptor, these compounds appear to open a novel cation channel (Bevan and Szolcsanyi, 1990); the resulting cation influx triggers a variety of biological responses including a local release of proinflammatory neuropeptides (efferent response), as well as pain perception (afferent response; see Holzer, 1991 for review). So far, two agents have been reported to block vanilloid-induced responses, namely RR (see Amann and Maggi, 1991 for review) and capsazepine (Dickenson and Dray, 1991; Urban and Dray, 1991; Bevan *et al.*, 1992). RR, an inorganic dye structurally unrelated to capsaicin, appears to be a nonspecific blocker of vanilloid actions because 1) RR also antagonizes the effects of other agents, for example protons (see Amann and Maggi, 1991 for review) and 2) RR fails to compete for specific [^{3}H]RTX binding sites thought to represent the vanilloid (capsaicin) receptor in DRG of the rat (Szallasi and Blumberg, 1990a). Moreover, the *in vivo* use of RR is restricted by its ability to induce convulsions of central nervous system origin (Tapia *et al.*, 1976). Capsazepine, a benzazepine derivative of capsaicin, by contrast, was shown to inhibit vanilloid-induced responses with a Schild plot suggestive of a competitive mechanism of action (Bevan *et al.*, 1992), was found not to provoke any biological response *per se* (Perkins and Campbell, 1992), and was reported to inhibit specific [^{3}H]RTX binding by rat DRG membranes (James *et al.*, 1992). A consistent view of the mechanism of action of capsazepine was, however, clouded by three findings: 1) capsazepine was reported to inhibit [^{3}H]RTX binding by rat DRG membranes with an unexpectedly poor potency (James *et al.*, 1992), 2) its potency to inhibit capsaicin- or RTX-induced responses was found to vary in the range of 10 to 1000 nM in different preparations (Dray *et al.*, 1991) and 3) capsazepine, like RR (Amann and Maggi, 1991), was found to block proton-induced "capsaicin-like" biological actions (Franco-Cereceda and Lundberg, 1992; Satoh *et al.*, 1992; Lou and Lundberg, 1992). Capsazepine has therefore been proposed to act on multiple targets (Franco-Cereceda and Lundberg, 1992; Lou and Lundberg, 1992). In the present report, we have explored the alternate possibility, *i.e.*, that different tissues of the rat express vanilloid receptors that recognize capsazepine with dissimilar potencies.

We have chosen the following tissues to evaluate the binding affinity of capsazepine: 1) DRG, containing the cell bodies of capsaicin-sensitive neurons (see Buck and Burks, 1986 for review); 2) spinal cord, containing the central terminals of capsaicin-sensitive neurons (see Buck and Burks, 1986 for review) and 3) two peripheral organs, the urinary bladder and the airways, in which capsaicin upon systemic administration produces a predominant inflammatory action mediated by a local release of proinflammatory neuropeptides (Saria *et al.*, 1983; Abelli *et al.*, 1991). In DRG membranes, capsazepine inhibited specific [^{3}H]RTX binding with a micromolar affinity; this affinity contrasts with the 0.1 to 0.2 μM potency of capsazepine to block capsaicin- and RTX-induced responses in cultured DRG neurons (Bevan *et al.*, 1992). It should be noted, however, that whereas we have used DRG membranes of adult rats for the binding studies, the cultured DRG neurons were obtained from neonates. Moreover, it is known that a greater proportion of DRG neurons are capsaicin-sensitive in the neonate than in the adult (Lawson and Harper, 1984). Therefore, it is complicated to correlate binding affinities determined in adult DRG to biological potencies measured in neonatal DRG. In spinal cord membranes, capsazepine displayed a binding affinity similar to that obtained in DRG. Capsazepine was shown to antagonize capsaicin-induced responses in the spinal cord-tail preparation of neonatal rat and mouse with submicromolar potency (Dickenson and Dray, 1991; Urban and Dray, 1991). Nevertheless, the comparison is hampered again by the species and age differences. In the urinary bladder of the adult rat, Maggi's group (1993) has found capsazepine to block capsaicin-induced responses at micromolar concentrations. In ac-

TABLE 1

Binding characteristics of vanilloid receptors expressed in different tissues of the rat

Values are mean ± S.E.M.; three determinations. For spinal cord and dorsal root ganglia, binding parameters were determined by fitting the allosteric Hill equation to the measured data; for urinary bladder and airway membranes, binding parameters were determined by the curvilinear regression program LIGAND (see under "Methods"). K_i values were calculated by using the equation of Cheng and Prusoff (see under "Methods").

Tissue	K_d	B_{max}	Hill number	Capsaicin (K_i)	Capsazepine (K_i)
	pM	*fmol/mg*		*μM*	
Spinal cord	20 ± 3	110 ± 20	1.7 ± 0.1	0.3 ± 0.1	4.0 ± 0.3
Dorsal root ganglia	15 ± 3	95 ± 10	1.8 ± 0.1	0.6 ± 0.4	3.5 ± 0.5
Urinary bladder*	30 ± 4	65 ± 14	0.95 ± 0.07	0.5 ± 0.2	5.0 ± 1.0
Airways	250 ± 26	76 ± 22	1.1 ± 0.05	0.14 ± 0.1	0.12 ± 0.02

* K_d, B_{max} and Hill number are from Szallasi *et al.* (1993).

cord, we have determined a K_i of 5 μM for capsazepine in rat urinary bladder membranes. As yet, the biological potency of capsazepine to inhibit capsaicin-induced responses in airways of the rat is unknown. The relatively high (0.1 μM) affinity of capsazepine to inhibit specific [^{3}H]RTX binding by rat airway membranes, nonetheless, predicts that capsazepine is a more powerful vanilloid antagonist in rat airways than in the urinary bladder.

At the theoretical level, our binding data may indicate the existence of three different vanilloid receptor subtypes in the rat (table 1): 1) the DRG-spinal cord-type that, on the one hand, binds RTX with high (15–20 pM) affinity and with apparent positive cooperativity (Szallasi and Blumberg, 1993) but, on the other hand, has a relatively low (micromolar) affinity for capsazepine; 2) the urinary bladder-type which binds RTX with high affinity (30 pM) in a noncooperative fashion (Szallasi *et al.*, 1993) and has relatively low affinity for capsazepine and 3) the airway type that binds RTX with a lower (260 pM) affinity in a noncooperative fashion but displays a higher (submicromolar) affinity for capsazepine. Interestingly, capsaicin does not seem to distinguish between these receptors. In addition to this intraspecies heterogeneity of the vanilloid (capsaicin) receptor, a growing body of evidence indicates the existence of marked interspecies differences both at the physiological and receptor level (see Buck and Burks, 1986; Holzer, 1991; Szallasi and Blumberg, 1992 for reviews).

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Title: **A comparison of capsazepine and ruthenium red as capsaicin antagonists in the rat isolated urinary bladder and vas deferens.**

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Abstract: 1. The ability of capsazepine, a recently developed capsaicin receptor antagonist, to prevent the effects of capsaicin on the rat isolated urinary bladder (contraction) and vas deferens (inhibition of electrically-evoked twitches) was compared to that of ruthenium red, a dye which behaves as a functional antagonist of capsaicin. 2. In the rat bladder, capsazepine (3-30 microM) produced a concentration-dependent rightward shift of the curve to capsaicin without any significant depression of the maximal response to the agonist. By contrast, ruthenium red (10-30 microM) produced a non-competitive type of antagonism, characterized by marked depression of the maximal response attainable. Similar findings were obtained in the rat isolated vas deferens in which capsazepine (10 microM) produced a rightward shift of the curve to capsaicin while ruthenium red (3 microM) depressed the maximal response to the agonist. 3. At the concentrations used to block the effect of capsaicin, neither capsazepine nor ruthenium red affected the contractile response of the rat urinary bladder produced by either neurokinin A or electrical field stimulation or the twitch inhibition produced by rat alpha-calcitonin gene-related peptide (alpha CGRP) in the vas deferens. 4. These findings provide additional evidence that both capsazepine and ruthenium red are valuable tools for exploration of the function of capsaicin-sensitive primary afferent neurones. The antagonism of the action of capsaicin by capsazepine is entirely consistent with the proposed interaction of this substance with a vanilloid receptor located on primary afferents, while the action of ruthenium red apparently involves a more complex, non-competitive antagonism.

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A comparison of capsazepine and ruthenium red as capsaicin antagonists in the rat isolated urinary bladder and vas deferens

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1 The ability of capsazepine, a recently developed capsaicin receptor antagonist, to prevent the effects of capsaicin on the rat isolated urinary bladder (contraction) and vas deferens (inhibition of electrically-evoked twitches) was compared to that of ruthenium red, a dye which behaves as a functional antagonist of capsaicin.

2 In the rat bladder, capsazepine (3–30 μM) produced a concentration-dependent rightward shift of the curve to capsaicin without any significant depression of the maximal response to the agonist. By contrast, ruthenium red (10–30 μM) produced a non-competitive type of antagonism, characterized by marked depression of the maximal response attainable. Similar findings were obtained in the rat isolated vas deferens in which capsazepine (10 μM) produced a rightward shift of the curve to capsaicin while ruthenium red (3 μM) depressed the maximal response to the agonist.

3 At the concentrations used to block the effect of capsaicin, neither capsazepine nor ruthenium red affected the contractile response of the rat urinary bladder produced by either neurokinin A or electrical field stimulation or the twitch inhibition produced by rat α-calcitonin gene-related peptide (αCGRP) in the vas deferens.

4 These findings provide additional evidence that both capsazepine and ruthenium red are valuable tools for exploration of the function of capsaicin-sensitive primary afferent neurones. The antagonism of the action of capsaicin by capsazepine is entirely consistent with the proposed interaction of this substance with a vanilloid receptor located on primary afferents, while the action of ruthenium red apparently involves a more complex, non-competitive antagonism.

Keywords: Capsaicin, primary afferent neurones; efferent function of sensory nerves; capsazepine; ruthenium red

Introduction

In recent years, much interest has developed in the pharmacological modulation of sensory neurone function (Maggi & Meli, 1988; Holzer, 1988; 1991; Maggi, 1991). A specific receptor on the cell membrane of primary afferent neurones has been recently discovered, which recognizes capsaicin and other natural pungent principles, including the ultrapotent capsaicin analogue, resiniferatoxin (Szallasi & Blumberg, 1990). This capsaicin or 'vanilloid' receptor fulfils several criteria to be considered the molecular target through which capsaicin and its congeners produce their specific actions on primary afferent neurones, which are characterized by long-lasting desensitization. Capsaicin also possesses non-specific actions (i.e. not restricted to primary afferent neurones) on nerves and smooth muscle which do not exhibit desensitization (Maggi & Meli, 1988; Holzer, 1991 for reviews). Studies from various laboratories have indicated that stimulation of the 'vanilloid' receptor on the membrane of primary afferent neurones is followed by the opening of a novel type of receptor-operated ion channel, which admits both sodium and calcium ions (Bevan & Szolcsanyi, 1991). It has recently been proposed that two different substances act as capsaicin antagonists, the inorganic dye ruthenium red (see Amann & Maggi, 1991 for review) which does not interact with the vanilloid receptor (Szallasi & Blumberg, 1990) and the benzazepine derivative, capsazepine (Bevan *et al.*, 1991; 1992; Dray *et al.*, 1991; Dickenson *et al.*, 1991; Urban & Dray, 1991), which was proposed as the first competitive antagonist of the capsaicin or vanilloid receptor.

Owing to the different mechanisms by which capsazepine and ruthenium red are thought to act as capsaicin antagonists, a direct comparison of these two drugs appeared of interest. With this aim we have chosen two preparations, the rat isolated urinary bladder and vas deferens, in which the selective action of capsaicin has been well characterized. In both preparations ruthenium red has been shown to antagonize the action of capsaicin by acting at a prejunctional site to inhibit the release of sensory neuropeptides which mediate the response to capsaicin (Maggi *et al.*, 1988a,b). The resultant contraction of the rat isolated bladder is mediated through the release of endogenous tachykinins (Maggi *et al.*, 1991); inhibition of electrically-evoked (field stimulation) twitches of the rat isolated vas deferens is mediated by the release of calcitonin gene-related peptide (CGRP) (Maggi *et al.*, 1987; Santicioli *et al.*, 1988). In both preparations a cumulative concentration-response curve to capsaicin has been used in order to determine the nature of the antagonism produced by capsazepine and ruthenium red.

Methods

Male albino rats of Wistar strain (body wt. 340–360 g) were stunned and bled. The whole urinary bladder and vasa deferentia were excised and placed in oxygenated (96% O_2 and 4% CO_2, pH 7.4) Krebs solution of the following composition (mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.5, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11.

Contractile responses of the urinary bladder and vas deferens (pars prostatica) were studied as described previously (Maggi *et al.*, 1987; 1991). Briefly, isometric tension

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recording was made from 1 cm long longitudinal strips of rat detrusor muscle and segments of the vas deferens 1.5 cm in length under a load of 10 mN (bladder) and 5 mN (vas deferens). In both preparations, electrical field stimulation was carried out by means of two wire platinum electrodes placed at the top and the bottom of the organ bath which were connected to a GRASS S88 stimulator. Square wave pulses (pulse width 0.5 ms, 60 V) were automatically delivered at a frequency of 0.01 Hz (bladder) and 0.2 Hz (vas deferens).

Experiments started after a 60 min equilibration period. In the rat bladder the contractile response to KCl (80 mM) was determined at 15 min intervals until reproducible responses were obtained. This response to KCl was used as an internal standard to evaluate the maximal response to capsaicin.

The effects of capsazepine and ruthenium red were investigated on a concentration-response curve produced by addition of capsaicin (10 nM–10 μM). In order to avoid or minimize capsaicin desensitization (see Results), the capsaicin concentration was increased by a factor of 10 for each successive dose of the cumulative nerve. Each dose of the cumulative curve was added when the effect of the preceding one had reached its maximum. Only one concentration-response curve to capsaicin was obtained in each preparation. Several preparations were made from the same animal, one of which served as control while the others received capsazepine or ruthenium red before capsaicin application.

Contact time for capsazepine and ruthenium red was 30 min. To check whether capsazepine and ruthenium red may have affected the capsaicin response at the postjunctional level, their effect was studied on the contraction of the rat urinary bladder produced by neurokinin A (1–30 nM) and inhibition of twitch contractions of the vas deferens produced by rat αCGRP (1–300 nM).

Statistical analysis of the data was performed by means of the Student's t test for paired or unpaired data or by analysis of variance, when applicable. All values in the text, figures and table are means ± s.e.mean.

Stock solutions were prepared as follows: capsaicin to given a final concentration of 10–100 mM, was dissolved in absolute ethanol and then diluted in water. Ruthenium red (1–10 mM, Aldrich) was dissolved in water. Capsazepine (10–100 mM) was dissolved in dimethylsulphoxide. Rat αCGRP and neurokinin A (Peninsula) were dissolved in water. The vehicles used to dissolve capsaicin (0.01% ethanol) or capsazepine (0.03% dimethylsulphoxide) had no effect on the preparations.

Results

General

Cumulative addition of capsaicin (10 nM–10 μM) with a ten fold increase in concentration between doses produced a concentration-dependent contraction of the rat isolated bladder. The maximal effect was observed at 1 μM, after which tension declined and 10 μM capsaicin did not evoke a larger response. The maximal response to capsaicin obtained with this protocol of cumulative addition (48 ± 4% of the response to 80 mM KCl, mean ± s.e.mean, $n = 10$) was slightly less than the response (55 ± 3% of KCl response, $n = 8$) observed when 1 μM capsaicin was added to the bath as a single concentration.

In the rat isolated vas deferens, cumulative addition of capsaicin (10 nM–10 μM) produced concentration-dependent inhibition of electrically-evoked twitches. The maximal inhibitory effect was observed with 1 μM capsaicin and was followed by a gradual recovery of the twitches amplitude. Addition of 1 μM capsaicin to the bath as a single dose produced a transient inhibitory effect (58 ± 4%, $n = 6$, cf. Maggi *et al.*, 1987) which was not different from that obtained in response to 1 μM capsaicin using a cumulative protocol (54 ± 3% inhibition, $n = 8$).

In the vas deferens, the twitch inhibition is ascribable to a specific action of capsaicin on sensory nerves and release of CGRP (Maggi *et al.*, 1987). In this preparation, at a threshold concentration of 10 μM, capsaicin produces an opposite effect, twitch enhancement (not shown), as originally described by Moritoki *et al.* (1987). This effect, which is repeatedly observed upon consecutive application of capsaicin in the same preparation, is ascribable to a nonspecific effect of the drug (i.e. not involving activation of sensory nerves, Maggi & Meli, 1988) and limits the possibility of exploring the effects of capsazepine over a large range of concentrations.

Effect of capsazepine and ruthenium red in the rat urinary bladder

Neither capsazepine nor ruthenium red affected spontaneous motility of the rat urinary bladder.

Capsazepine (3–30 μM) produced a concentration-dependent rightward shift of the concentration-response curve to capsaicin in the rat bladder (Figure 1). In the presence of 3 μM capsazepine, the response to a threshold concentration of capsaicin (10 nM) was blocked but the maximal response to capsaicin was obtained at the same concentration (1 μM) as in controls. Ten and 30 μM capsazepine produced a further inhibition of the response to capsaicin and in this case the maximal response to the agonist was obtained at 10 μM capsaicin (Figure 1).

The maximal response to capsaicin in the presence of 3–30 μM capsazepine was not significantly different from that found in control experiments (Table 1).

Ruthenium red (3–10 μM) produced a concentration-dependent inhibition of the response to capsaicin in the rat bladder. As can be noted from curves presented in Figure 1b, the maximal response to capsaicin in the presence of ruthenium red was depressed as compared to controls.

Neither capsazepine (10–30 μM) nor ruthenium red (30 μM) significantly affected contractions produced by neurokinin A (Figure 1c and d) nor twitch contractions of the rat bladder produced by electrical field stimualtion at 0.01 Hz (Table 1).

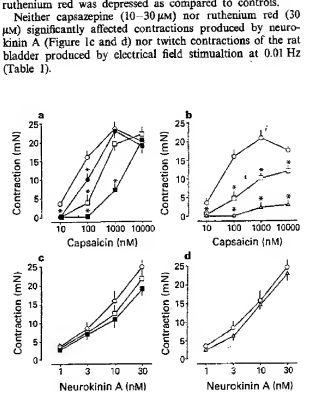

Figure 1 Effect of capsazepine (CPZ, a,c) or ruthenium red (RR, b,d) on concentration-response curve for capsaicin-induced (a,b) or neurokinin A-induced (c,d) contraction of the rat isolated urinary bladder. In (a) and (c): control (○); CPZ 3 μM (●); 10 μM (□); 30 μM (■). In (b) and (d): control (○); RR 10 μM (□); 30 μM (△). Each value is mean ± s.e.mean (vertical lines) of 4–12 experiments.

Table 1 Effect of capsazepine and ruthenium red on contractile responses of the rat isolated urinary bladder to various agents

	Response to EFS (mN)	*Response to NKA* (30 nM) (mN)	*Maximal response to capsaicin* (mN)
Controls	13.8 ± 1.5	25 ± 2	23 ± 2
Capsazepine 3 μM	14.0 ± 2.5	NT	23 ± 6
Capsazepine 10 μM	13.2 ± 4.0	21 ± 2	22 ± 4
Capsazepine 30 μM	11.0 ± 1.2	19 ± 3	20 ± 4
Ruthenium red 10 μM	11.7 ± 3.0	NT	12 ± 2*
Ruthenium red 30 μM	12.5 ± 2.0	23 ± 2	3 ± 1*

Contractile response to electrical field stimulation (EFS, 0.01 Hz, 0.5 ms pulse width, maximal voltage), neurokinin A (NKA) or capsaicin are mean ± s.e.mean.
Number of experiments is 9–12 in the control group and 4–6 for each group with capsazepine or ruthenium red.
Significantly different from control: *$P < 0.05$.

Effect of capsazepine and ruthenium red in the rat vas deferens

Capsazepine (10 μM) produced a slight (10–20%) enhancement of twitch contractions of the rat isolated vas deferens to electrical stimulation (0.2 Hz, 0.5 ms pulse width, maximal voltage). Capsazepine (10 μM) produced a parallel rightward shift of the concentration-response curve to capsaicin, with no apparent depression of maximal inhibitory response (Figure 2). In the presence of 10 μM capsazepine, the response to 10–100 nM capsaicin was virtually abolished and a concentration of 10 μM capsaicin was required to produce a transient maximal twitch inhibition. This effect was followed by a prompt recovery of twitch amplitude, possibly related to the nonspecific potentiating effect produced by capsaicin at 10 μM, as described above.

Ruthenium red (3 μM) inhibited twitch amplitude by 38 ± 5% ($n = 4$). In the presence of ruthenium red, the concentration-response curve to capsaicin was shifted to the right and maximum effect was depressed compared to controls (Figure 2). In Figure 2, the inhibitory effect of capsaicin in the presence of ruthenium red (and rat αCGRP, see below) is expressed as % reduction of the new baseline. The maximal twitch inhibition produced by 10 μM capsaicin in the presence of 3 μM ruthenium red (21 ± 3% inhibition) was significantly less than the maximal response obtained in either control preparations (55 ± 4% inhibition at 1 μM capsaicin) or in the presence of capsazepine (54 ± 3% inhibition at 10 μM capsaicin).

Rat αCGRP (10 nM–0.3 μM) produced a concentration-dependent inhibition of twitch amplitude in the rat isolated vas deferens, as described previously (Maggi *et al.*, 1987). The inhibitory effect of CGRP was unaffected by capsazepine (10 μM) or ruthenium red (3 μM) (Figure 2b).

Discussion

In this study we have used a cumulative protocol of capsaicin administration to investigate capsaicin antagonism by capsazepine and ruthenium red. It is well known that capsaicin produces a functional desensitization of primary afferent neurones. In descriptive terms, this means that, after application of the drug, the primary afferents become insensitive to further applications of capsaicin itself as well as to other agents which stimulate the primary afferent neurones. The capsaicin desensitization phenomenon is concentration-, time- and temperature-dependent (e.g. Amann, 1990) and probably involves different mechanisms such as a true desensitization (tachyphylaxis) of the vanilloid receptor (Dray *et al.*, 1989; Maggi *et al.*, 1990), blockade of transmitter release through the inactivation of voltage-sensitive calcium channels (Bleakman *et al.*, 1990; Docherty *et al.*, 1991) and degenerative changes in primary afferents owing to the marked influx of cations produced by capsaicin (Bevan & Szolcsanyi, 1990; Maggi, 1991; Holzer, 1991).

To overcome this problem, increasing concentrations of capsaicin were added to the bath with a ten fold increase in concentration at each step, while the effect of antagonists was compared to control curves obtained in preparations from

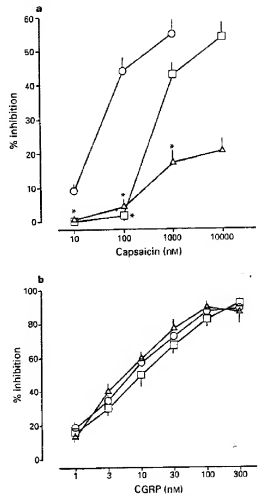

Figure 2 Effect of capsazepine (CPZ) or ruthenium red (RR) on concentration-response curve for capsaicin-induced (a) or rat α-calcitonin gene-related peptide (αCGRP)-induced (b) inhibition of twitch contractions of the rat isolated vas deferens: control (○); CPZ 10 μM (□); RR 3 μM (△). Each value is mean ± s.e.mean (vertical lines) of 4–12 experiments.

the same animal. The maximal response produced by cumulative application of capsaicin was only slightly less (bladder) or not significantly different (vas deferens) from that produced by the administration of a 1 μM single dose of capsaicin.

A drawback of the technique we have used is that the whole curve obtained with the agonist, either in the absence or presence of antagonists, is very steep and the few experimental points obtained in this way do not lend themselves to a conventional analysis for establishing whether competitive antagonism occurred (i.e. measurement of ED_{50}s, dose-ratios and Schild plots). In spite of this limitation, the different nature of antagonism by capsazepine and ruthenium red was very evident in both preparations investigated. Capsazepine essentially produced a rightward shift of the concentration-response curve to capsaicin without any significant depression of the maximum response. The failure of capsazepine to modify significantly the contractile response to neurokinin A in the bladder or to CGRP in the vas deferens ruled out a postjunctional action to inhibit the effect of endogenous neuropeptides released by capsaicin. Likewise, the failure of capsazepine to affect the amplitude of twitch contractions in both the bladder and the vas deferens excludes a nonspecific neuronal depressant action.

Overall, the present results are in good agreement with the competitive antagonist character of capsazepine action at the vanilloid receptor, as demonstrated in other test systems (Bevan *et al.*, 1991; 1992; Dray *et al.*, 1991).

In sharp contrast, ruthenium red displaced the curve to capsaicin in a manner that is indicative of non-competitive antagonism. The ability of ruthenium red to antagonize the action of capsaicin in the rat bladder and vas deferens at a prejunctional site of action was demonstrated in previous studies (Maggi *et al.*, 1988a,b) but the nature of antagonism (competitive vs. noncompetitive) had not been investigated. In contrast to capsazepine, ruthenium red at the concentration required to antagonize capsaicin, may have a neuronal depressant action not restricted to primary afferent neurones. This aspect of ruthenium red pharmacology, already noted in previous investigations (e.g. Chahl, 1989), is further confirmed here by its depressant action on twitch contractions of the isolated vas deferens. This effect of ruthenium red is however very variable from one preparation to another and cannot account for the ability of the dye to act as functional capsaicin antagonist (Amann & Maggi, 1991). Evidence has been presented (Dray *et al.*, 1990; Bleakman *et al.*, 1990) indicating that ruthenium red acts at the level of the cell membrane of primary afferent neurones to prevent the opening of the vanilloid receptor-operated cation channel. Although the molecular mechanism by which ruthenium red acts as a functional capsaicin antagonist has not yet been established, the non-competitive antagonism seen in this study is in keeping with its proposed site of action at a step beyond the occupation of the vanilloid receptor by the agonist.

The results of the present study, while confirming that both capsazepine and ruthenium red are useful tools to explore sensory neurone function, indicate that these two agents act at distinct steps in the sequence of events which is activated by capsaicin application. The different nature of the antagonism by capsazepine and ruthenium red is strongly reminiscent of the manner in which other drugs (e.g. bicuculline and picrotoxin at the $GABA_A$ receptor) act to inhibit receptor-operated ion channels and this parallel may be kept in mind in planning further experiments analysing the pharmacology of the vanilloid receptor and functional responses coupled to its activation.

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High-Affinity Partial Agonists of the Vanilloid Receptor

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ABSTRACT

The vanilloid receptor VR1 is a polymodal nociceptor sensitive to capsaicin, protons, and heat. Because VR1 represents an attractive therapeutic target for conditions ranging from long-term pain to bladder hyperreflexia, we and other groups have sought to develop novel ligands with enhanced potencies and novel pharmacological properties. Here, we characterize two compounds, *N*-[2-(3,4-dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[4-(methylsulfonylamino)benzyl]thiourea (JYL827) and *N*-(4-*tert*-butylbenzyl)-*N*′-[3-methoxy-4-(methylsulfonylamino)benzyl]thiourea (JYL1511), that function as partial agonists for rat VR1 heterologously expressed in Chinese hamster ovary cells. Both compounds showed substantially enhanced potency, inhibiting [^{3}H] resiniferatoxin binding with *K*$_i$ values of 29.3 ± 7.6 and 50.4 ± 16.5 nM, respectively, compared with 1810 ± 270 nM for capsaicin. The compounds showed different extents of partial agonism, 6.8 ± 0.7% and 17.4 ± 0.6%, respectively, and the expected corresponding degrees of partial antagonism (93.9 ± 0.9 and 84.1 ± 3.2%, respectively). Their IC$_{50}$ values for antagonism of $^{45}Ca^{2+}$ uptake in response to capsaicin were 67.3 ± 24.9 nM and 3.4 ± 0.5 nM, respectively. Protons, temperature, and protein kinase C all function as coactivators/modulators of rVR1. All enhanced the extent of partial agonism of JYL827 and JYL1511. Thus, at pH 5.5, for example, the extents of partial agonism increased to 54.9 ± 2.5% and to 90.7 ± 1.7%, respectively, relative to the response elicited by 300 nM capsaicin. The extents of partial antagonism decreased correspondingly. Compounds such as JYL827 and JYL1511 now permit exploration of the potential utility of partial agonists of rVR1 in animal models. Our results emphasize, moreover, the strong dependence of such partial agonists on other modulators of rVR1 and predict that their biological behavior will depend strongly on biological context.

The vanilloid receptor VR1 has attracted great attention, because of both its biological function and its therapeutic potential. VR1, also called vanilloid receptor type 1, a member of the transient receptor potential family of ion channels, is a modestly calcium-selective ion channel located in C-fiber and Aδ sensory neurons as well as in a growing number of other sites such as the central nervous system or the bladder (Szallasi, 2001). VR1 functions as an integrative transducer for a range of nociceptive signals, including heat, protons, endogenous ligands, such as lipoxygenase products or anandamide, and exogenous compounds such as capsaicin or resiniferatoxin (Julius and Basbaum, 2001). Activation of VR1 by capsaicin may be followed by subsequent loss of responsiveness, depending on dose, duration of application, and other conditions, and reflects a combination of overlapping mechanisms, such as dephosphorylation or calcium toxicity to the neurons (Szallasi and Blumberg, 1999). This desensitization/defunctionalization by capsaicin has been exploited to treat a variety of conditions in which C-fiber sensory neurons are involved, such as pain associated with arthritis, cystitis, human immunodeficiency virus, and diabetic neuropathy (Robbins, 2000).

Although capsaicin has made it possible to identify an exciting series of potential therapeutic applications, its utility has been limited by its somewhat modest potency, by the initial pain occasioned upon initial application, and by its metabolic lability (Szallasi, 2001). Attention has therefore been directed at the development and characterization of novel analogs of capsaicin. Although still in the early stages, much progress has been made.

Ligands have been identified with much greater potency for VR1 than that displayed by capsaicin. Resiniferatoxin (RTX), isolated from *Euphorbia resinifera*, is a natural product in which the alkyl C-region of capsaicin is replaced with a tricyclic diterpene structurally related to those found in the phorbol esters. RTX binds to rVR1 with an affinity 4 orders of

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ABBREVIATIONS: RTX, resiniferatoxin; JYL1511, *N*-(4-*tert*-butylbenzyl)-*N*′-[3-methoxy-4-(methylsulfonylamino)benzyl]thiourea; JYL827, *N*-[2-(3,4-dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[4-(methylsulfonylamino)benzyl]thiourea; CHO, Chinese hamster ovary; CHO/rVR1 cell, Chinese hamster ovary cells transfected with rVR1; PBS, phosphate-buffered saline; DPBS, Dulbecco's modified PBS with Ca^{2+} and Mg^{2+}; MES, 2-[*N*-morpholino]ethanesulfonic acid; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; rVR1, cloned rat vanilloid receptor subtype-1.

magnitude stronger than that of capsaicin (Szallasi et al., 1999a). Incorporating elements of the postulated pharmacophoric groups provided by the diterpene moiety of RTX into synthetic capsaicin analogs, we have characterized compounds with binding affinities for rVR1 up to 280-fold stronger than that of capsaicin (Lee et al., 2001a).

Antagonists for VR1 have also been developed. Capsazepine, the most extensively characterized, is a competitive antagonist of capsaicin with affinity similar to that of capsaicin (Bevan et al., 1992). A problem has been its limited selectivity; it also blocks nicotinic cholinergic receptors, voltage dependent calcium channels, and purinergic receptors at concentrations comparable with those at which it is active on VR1 (Docherty et al., 1997; Liu and Simon, 1997; Wardle et al., 1997). 5-Iodo-4-hydroxy-3-methoxy RTX shows markedly enhanced potency, with an IC_{50} for rat VR1 expressed in *Xenopus laevis* oocytes of 3.9 nM (40-fold stronger than that of capsazepine in this system) (Wahl et al., 2001). *N*-(4-*tert*-butylbenzyl)-*N*′-[3-fluoro-4-(methylsulfonylamino)-benzyl]thiourea (compound 1) (Suh et al., 2002a), one of a series of synthetic capsaicin analogs with antagonistic activity, gives an IC_{50} for rat VR1 expressed in CHO cells of 9.2 nM (60-fold stronger than that of capsazepine in this system) (Wang et al., 2002).

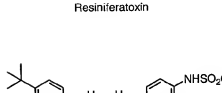
Metabolism can be restricted, with marked enhancement in activity upon oral administration, as exemplified by *N*-(4-(2-aminoethoxy)-3-methoxybenzyl)-*N*′-(4-*tert*-butyl-benzyl)thiourea. This compound was 2-fold more potent than capsaicin for inducing Ca^{2+} influx in cultured cells but 640-fold more potent than capsaicin in the mouse tail-flick assay upon oral administration (Wrigglesworth et al., 1996).

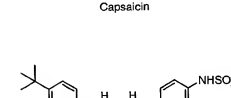
Finally, different endpoints of biological response to vanilloids have been shown to be separable, at least partially. Of particular interest is that compounds such as olvanil or RTX show reduced pungency relative to their ability to desensitize (Szallasi and Blumberg, 1989; Liu et al., 1997). Likewise, effects on inflammation can be dissociated from those on thermoregulation (Szallasi et al., 1999b).

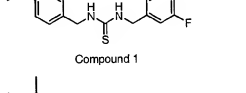
An ongoing effort of this group has been to exploit the postulated pharmacophoric groups of RTX, as well as other strategies, to generate capsaicin analogs with enhanced potency and novel properties (Lee et al., 1999, 2001a,b; 2002). We have identified multiple derivatives that show partial efficacy relative to capsaicin in their ability to induce calcium uptake in cells expressing rVR1. We characterize here in detail the activity of two compounds, selected because of somewhat different levels of partial efficacy. JYL827 illustrates a compound with quite limited agonism under standard conditions, which might cause it mistakenly to be treated as a full antagonist; JYL1511 illustrates a compound with a higher level of agonism. These compounds, the structures of which were initially described elsewhere (Suh et al., 2002a,b), are shown to function as partial agonists, and the extent of their partial agonism depends on the presence of coactivators such as protons, heat, or activation of protein kinase C.

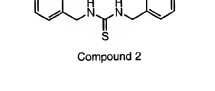
Materials and Methods

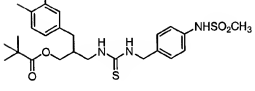
Materials. JYL1151 and JYL827 were synthesized as described elsewhere (Suh et al., 2002a,b). The structures of these compounds and of related structures are shown in Fig. 1. [^{3}H]RTX (37 Ci/mmol) was provided by PerkinElmer Life Sciences (Boston, MA). $^{45}Ca^{2+}$ was from ICN Biomedicals, Inc. (Irvine, CA). Nonradioactive RTX, capsaicin, and capsazepine were purchased from Alexis Corp (San Diego, CA).











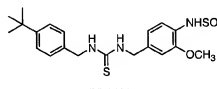


Fig. 1. Structures of JYL827 and JYL1511. Also shown for comparison are the structures of capsaicin and two full antagonists (Suh et al., 2002a; Wang et al., 2002). Compound 1, *N*-(4-*tert*-butylbenzyl)-*N*′-[3-fluoro-4-(methylsulfonylamino)benzyl]thiourea; compound 2, *N*-(4-*tert*-butylbenzyl)-*N*′-[4-(methylsulfonylamino)benzyl]thiourea.

Preparation and Subculture of Cells Stably Expressing Rat VR1. Chinese hamster ovary (CHO) cells stably transfected with rat VR1 in a pTet off regulatory system were described previously (Szallasi et al., 1999a). In this system, expression of the rVR1 is repressed in the presence of tetracycline but is induced upon removal of the antibiotic. The cells were maintained in medium supplemented with tetracycline (1 μg/ml) (Szallasi et al., 1999a). Cells used for assays were grown in culture medium without tetracycline for 48 h before use. For radioligand binding experiments, cells were seeded in T75 cell culture flasks in the culture media with tetracycline (1 μg/ml) and G-418 (0.25 mg/ml). After 2 days, the culture medium was changed to medium without tetracycline and the cells were grown for an additional 48 h to induce rVR1 expression. The flasks were washed with PBS and the cells harvested in PBS containing 5 mM EDTA. The cells were pelleted by gentle centrifugation and stored at −20°C until assayed. For assay of $^{45}Ca^{2+}$ uptake, cells were seeded into 24-well plates in media with tetracycline (1 μg/ml) and G-418 (0.25 mg/ml). After 1 day, the culture medium was changed to medium without tetracycline and the cells were grown for an additional 48 h to induce VR1 expression. For calcium imaging, cells were grown on glass coverslips (25 mm).

Competition Binding Assay. Binding studies with [^{3}H]RTX were carried out as described previously with minor modifications (Wang et al., 2002). Binding assay mixtures were set up on ice and contained 80 pM [^{3}H]RTX, various concentrations of competing ligands, 0.25 mg/ml BSA (Cohn fraction V), and 5×10^5 to 10^5 CHO/rVR1 cells. The final volume was adjusted to 450 μl with DPBS containing Ca^{2+} and Mg^{2+} (Invitrogen, Gaithersburg, MD) and 0.25 mg/ml bovine serum albumin. Nonspecific binding was determined in the presence of 100 nM nonradioactive RTX. The binding reaction was initiated by transferring the assay mixtures to a 37°C water bath and was terminated after a 60-min incubation period by cooling the tubes on ice. Nonspecific binding was reduced by addition of 200 μg of bovine glycoprotein fraction VI (α-glycoprotein) (ICN, Costa Mesa, CA) to each tube. Membrane-bound RTX was separated from free RTX by pelleting the membranes in a model 12 Microfuge (15 min, maximal velocity; Beckman Coulter, Fullerton, CA); the tips of the tubes containing the pellets were cut off, and the radioactivity was determined by scintillation counting. Equilibrium binding parameters (K_i, B_{max}, and cooperativity) were determined by fitting the Hill equation to the measured values with the aid of the program Origin 6.0 (OriginLab Corp., Northampton, MA).

$^{45}Ca^{2+}$ Uptake. CHO/rVR1 cells were incubated for 5 min at 37°C or as indicated with 0.2 μCi/well $^{45}Ca^{2+}$ in the presence of serum-free DMEM, 0.25 mg/ml bovine serum albumin, and various concentrations of the different compounds. To determine the pH dependence of $^{45}Ca^{2+}$ uptake, cells were incubated for 5 min at 37°C with 0.2 μCi/well $^{45}Ca^{2+}$ in the presence of DPBS, supplemented with 0.25 mg/ml bovine serum albumin and various concentrations of the different compounds, adjusted to the indicated pH with 1 M MES (Sigma, St. Louis, MO). After incubation, cells were washed 3 times with DPBS and lysed in 400 μl/well of radioimmunoprecipitation assay buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% SDS) for 20 min. Aliquots of the solubilized cell extracts were counted in a liquid scintillation counter.

Imaging of Intracellular Calcium Levels [Ca^{2+}]$_i$. Cells grown on coverslips were loaded with Fura-2 AM (10 μM) (Molecular Probes, Eugene, OR) for 10 min at 37°C and an additional 50 min at room temperature (for CHO/rVR1 cells), washed, and then incubated at room temperature for at least an additional hour. Coverslips were placed in a chamber at room temperature. Images of Fura-2–loaded cells with the excitation wavelength alternating between 340 and 380 nM were captured using a Cohu 4915 low-light CCD camera on an InCyt Dual-Wavelength Fluorescence Imaging and Photometry System (Intracellular Imaging Inc., OH). The ratio of fluorescence intensity at the two wavelengths was calculated.

Results

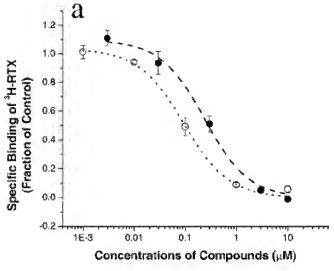
JYL827 and JYL1511 Potently Bind to Rat VR1. We have previously demonstrated that ligand binding to rat VR1 heterologously expressed in CHO cells or HEK293 cells closely resembles that characterized in rat dorsal root ganglion neurons (Szallasi et al., 1999a). We have therefore used the CHO/rVR1 system for determination of ligand structure activity relations. JYL827 and JYL1511 inhibited [^{3}H]RTX binding to rat VR1 with K_i values of 29.3 ± 7.6 nM (n = 6 experiments) and 50.4 ± 16.5 nM (n = 3 experiments), respectively (Fig. 2a). For comparison, the K_i of capsaicin under these conditions was 1810 ± 270 nM (J. Lee, J. Lee, M. Kang, M. Y. Shin, J. M. Kim, S. U. Kang, J. O. Lim, H. K. Choi, Y. G. Suh, H. G. Park, et al., submitted). The compounds were thus 60- and 35-fold more potent, respectively, than capsaicin for binding to rVR1.

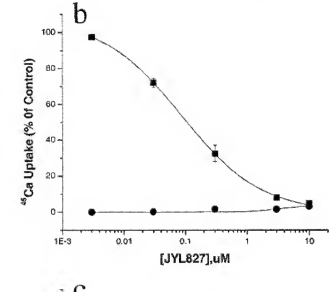
JYL827 and JYL1511 Function as Partial Agonists/Partial Antagonists on Rat VR1. We evaluated agonism by the activation of $^{45}Ca^{2+}$ uptake by CHO/rVR1 cells upon incubation with compounds for 5 min. The levels of $^{45}Ca^{2+}$ uptake were compared with that induced by a saturating concentration of capsaicin (300 nM under these conditions). JYL827 and JYL1511 induced 6.8 ± 0.7% (n = 7 experiments) and 17.4 ± 0.6% (n = 6 experiments) of the level of $^{45}Ca^{2+}$ uptake induced by capsaicin. The EC_{50} values were 35.5 ± 4.2 nM (n = 4 experiments) and 32.4 ± 5.3 nM (n = 3 experiments), respectively (Fig. 2, b and c).

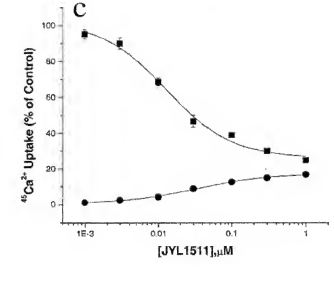
Although partial efficacy of these compounds might imply that they function as partial agonists on rat VR1, an alternative is that the compounds have difficulties crossing the plasma membrane to reach the ligand binding site of VR1, which is on the inner face of the membrane (Jung et al., 1999). We therefore examined the ability of the compounds to antagonize $^{45}Ca^{2+}$ uptake induced by 50 nM capsaicin when the compound and capsaicin were added simultaneously. We used the 50 nM concentration of capsaicin, approximately its EC_{50}, to minimize the rightward shift in antagonist dose response curves caused by competition with the capsaicin. Under these conditions, capsaicin induction of $^{45}Ca^{2+}$ uptake was antagonized both by JYL827 and by JYL1511 (Fig. 2, b and c). The levels of inhibition by the compounds of the $^{45}Ca^{2+}$ uptake induced by capsaicin were complementary to the levels of stimulation of $^{45}Ca^{2+}$ uptake by the compounds alone. Thus, JYL827 inhibited by 93.9 ± 0.9% (n = 7 experiments) and JYL1511 inhibited by 84.1 ± 3.2% (n = 6 experiments), giving total values for the percentage of agonism plus antagonism of 101 and 102%, respectively, as expected for a mechanism of partial agonism. The IC_{50} value of JYL827 for antagonism of $^{45}Ca^{2+}$ uptake induced by capsaicin was 67.3 ± 24.9 nM (n = 4 experiments); the IC_{50} value of JYL1511 was 3.4 ± 1.0 nM (n = 3 experiments). For JYL827, the EC_{50} and IC_{50} values show good agreement. For JYL1511, the EC_{50} value is higher (less potent), presumably reflecting the difficulties of quantitation when the extent of agonism is low. We conclude that JYL827 and JYL1511 are partial agonists/partial antagonists, representing different extents of partial agonism.

Activity of JYL827 and JYL1511 on CHO/RVR1 Cells as Evaluated by Calcium Imaging. Calcium imaging provides an alternative measure for VR1 responsiveness. In the CHO/rVR1 system, a maximally effective dose of JYL827 (3

μM) caused a barely measurable increase in $[Ca^{2+}]_i$, and JYL1511 (3 μM) caused an intermediate response, although clearly much less than that induced by 300 nM capsaicin (Fig. 3, a–c). These results are consistent with the results determined by $^{45}Ca^{2+}$ uptake.







VR1 Expression Level Governs the Efficacy of Partial Agonists. Receptor density is known to be a factor strongly influencing the efficacy of ligands in both tissue and recombinant systems, particularly in systems under conditions of spare receptors (Kenakin, 1997). In our CHO/rVR1 system, the expression of rVR1 is repressed by tetracycline and is induced upon removal of the antibiotic. By varying the concentration of tetracycline (0, 1, 5, 10 μg/ml) in the maintaining medium, we could obtain different levels of receptor expression in the CHO/rVR1 cells, which we measured by binding of [^{3}H]RTX under saturating conditions (Fig. 4a). With the increase in the expression of rVR1, JYL827 shifted from a full antagonist to a partial agonist (0 to 23 $\pm$ 0.56% of the level of $^{45}Ca^{2+}$ uptake induced by 300 nM capsaicin, n = 3 experiments) (Fig. 4b). For JYL1511, partial agonism increased from 17.4 $\pm$ 0.6 to 59 $\pm$ 1.2% (n = 3 experiments) relative to capsaicin (Fig. 4c). The maximum response of the full agonist capsaicin also increased as the level of expression of rat VR1 increased, but the magnitude was much less than that of the partial agonists (data not shown). We conclude that the level of VR1 expression has an important effect on whether a compound appears as an antagonist or partial agonist as well as on the degree of partial agonism.

Temperature Affects the Efficacy of Partial Agonists. Elevated temperature is a potent activator of VR1 and is a potentiator of the action of capsaicin on VR1 (Tominaga et al., 1998). We therefore examined the effect of temperature on the response of the CHO/rVR1 cells to JYL827 and JYL1511. In contrast to the full antagonists of capsaicin action that we described previously, neither JYL827 nor JYL1511 antagonized the $^{45}Ca^{2+}$ uptake induced by elevated temperature (Wang et al., 2002). Rather, increasing temperature enhanced the extent of partial agonism by the compounds (Fig. 5a). For JYL827, the extent of partial agonism increased from 6.8 $\pm$ 0.7% (n = 7 experiments) at 37°C to 21.3 $\pm$ 1.8% (n = 3 experiments) at 44°C. For JYL1511, which has more efficacy as an agonist, its extent of partial agonism increased from 17.4 $\pm$ 0.6% (n = 6 experiments) at 37°C to 37.7 $\pm$ 3.1% (n = 3 experiments) at 44°C. The extents of $^{45}Ca^{2+}$ uptake were expressed relative to those induced by capsaicin (300 nM) at the same temperature. Consistent with the expected behavior for partial agonists, increasing temperature reduced the extent of partial antagonism by the compounds (Fig. 5b). For JYL827, the extent of partial antagonism decreased from 93.9 $\pm$ 0.9% (n = 7 experiments) at 37°C to 74.8 $\pm$ 4.0% (n = 3 experiments) at 44°C. For JYL1511, which has less efficacy as an antagonist, the extent of partial antagonism decreased from 84.1 $\pm$ 3.2% (n = 6

Fig. 2. Activity of JYL827 and JYL1511 on CHO/rVR1 cells. A, inhibition of specific [^{3}H]RTX binding. (○), JYL827; (●), JYL1511. Points represent the mean $\pm$ S.E.M. of triplicate determinations in single experiments. All experiments repeated at least three times. b, activity of JYL827 for (●) induction of $^{45}Ca^{2+}$ uptake and for (■) inhibition of $^{45}Ca^{2+}$ uptake induced by capsaicin. For evaluation of JYL827 as an agonist, $^{45}Ca^{2+}$ uptake in excess of that for the medium control was determined for the indicated concentrations of JYL827 and was expressed relative to that by a maximally effective dose of capsaicin (300 nM). For evaluation of JYL827 as an antagonist, $^{45}Ca^{2+}$ uptake in excess of that for the medium control was determined for the indicated concentrations of JYL827 in the presence of 50 nM capsaicin. Points represent mean values of four determinations in single, representative experiments; error bars indicate S.E.M. All experiments were repeated at least two additional times with similar results. c, activity of JYL1511 for induction (●) and for inhibition (■) of $^{45}Ca^{2+}$ uptake induced by capsaicin. Analysis was as described for JYL827.

experiments) at 37°C to 61.2 ± 3.5% (n = 3 experiments) at 44°C. The extents of $^{45}Ca^{2+}$ uptake were expressed relative to those induced by capsaicin (300 nM) at the same temperature. For capsaicin, the absolute levels of $^{45}Ca^{2+}$ uptake were similar at all three temperatures. We conclude that the extent of partial agonism and partial antagonism is not an intrinsic characteristic of the ligand but rather depends on other coregulators, in this case temperature.

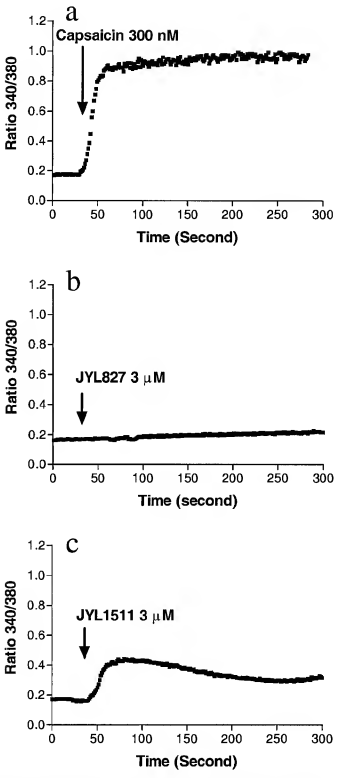


Fig. 3. Capsaicin and partial agonists evoked calcium mobilization in CHO/rVR1 cells as determined by calcium imaging. CHO/rVR1 cells were treated with capsaicin, JYL827, or JYL1511 (indicated by arrows). Points represent the averaged signal from a minimum of 16 cells imaged simultaneously. Each experiment was repeated at least an additional two times with similar results on independently cultured cells.

Protons Enhanced the Efficacy of Partial Agonists. Protons represent another class of well-characterized agonists for VR1 and are potentiators of the action of capsaicin on VR1 (Tominaga et al., 1998). They are of particular interest because of the physiological role that acidosis is believed to play in inflammatory pain. We have previously shown that

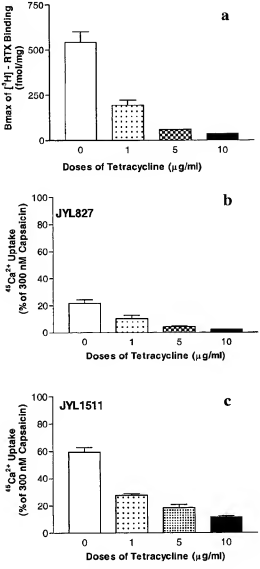


Fig. 4. $^{45}Ca^{2+}$ uptake evoked by partial agonists as a function of rat VR1 expression level. The CHO/rVR1 cells were cultured for 2 days in the presence of the indicated concentrations of tetracycline to give different levels of expression of rVR1. a, to compare levels of rVR1 expression, B_{max} values for [^{3}H]RTX binding (fmol/mg) were determined. The values were calculated from complete saturation binding curves in single, representative experiments. The experiment was repeated two additional times with similar results. b and c, $^{45}Ca^{2+}$ uptake induced by JYL827 (3 μM) and JYL1511 (3 μM). Levels of $^{45}Ca^{2+}$ uptake over baseline were expressed relative to that induced by 300 nM capsaicin. Points represent mean values of four determinations in single, representative experiments; error bars indicate S.E.M. All experiments were repeated at least two additional times with similar results.

different complete antagonists for capsaicin action on rat VR1 may fully or partially block the $^{45}Ca^{2+}$ uptake induced by a reduction in pH (Wang et al., 2002). We therefore examined $^{45}Ca^{2+}$ uptake in response to JYL827 and JYL1511 as the pH was reduced from pH 7.4 to 5.5 (Fig. 6). In contrast to the full antagonists of capsaicin action we described earlier, neither JYL827 nor JYL1511 antagonized the induction of $^{45}Ca^{2+}$ uptake by the lower pH. JYL827 stimulated $^{45}Ca^{2+}$ uptake, relative to that induced by capsaicin, by 6.8 ± 0.7% (n = 7 experiments) at pH 7.4 and by 54.9 ± 2.5% at pH 5.5 (n = 3 experiments). Conversely, the extent of partial antagonism of capsaicin induced $^{45}Ca^{2+}$ uptake decreased from 93.9 ± 0.9% (n = 7 experiments) at pH 7.4 to 42.9 ± 1.9% (n = 3 experiments) at pH 5.5.

JYL1511, as expected, showed a greater degree of agonism and less antagonism than did JYL827 at all pH values. The extent of partial agonism increased from 17.4 ± 0.6% (n = 6 experiments) at pH 7.4 to 90.7 ± 1.7% at pH 5.5 (n = 3 experiments), whereas the extent of partial antagonism decreased from 84.1 ± 3.2% (n = 6 experiments) at pH 7.4 to 8.0 ± 3.1% (n = 3 experiments) at pH 5.5. The extents of $^{45}Ca^{2+}$ uptake were expressed relative to those induced by capsaicin (300 nM) at the same pH. Once again, our results demonstrate that compounds cannot be regarded simply as antagonists or agonists for VR1; rather, their actions depend on the context in which VR1 is present.

The PKC Intracellular Signaling Pathway Can Potentiate Efficacy of Partial Agonists. It is well known that protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat, and anandamide (Vellani et al., 2001; Crandall et al., 2002). Here, we examined the effect of the PKC activator PMA on the potency and efficacy of JYL827 and JYL1511 (Fig. 7). PMA (100 nM) increased the extent of partial agonism of JYL827 from 6.8 ± 0.6% (n = 7 experiments) to 17.1 ± 0.7% (n = 3 experiments) and that of JYL1511 from 17.4 ± 0.6% (n = 6 experiments) to 27.8 ± 1.0% (n = 3 experiments). Conversely, PMA decreased the extent of partial antagonism of JYL827 from 93.9 ± 0.9% (n = 7 experiments) to 82.8 ± 0.7% (n = 3 experiments) and that of JYL1511 from 84.1 ± 3.2% (n = 6 experiments) to 72.2 ± 1% (n = 3 experiments). We conclude that intracellular signaling pathways can also control the degree of partial agonism and antagonism.

Synergistic Effect of Coactivators on the Efficacy of Partial Agonists. We have described above that individual coactivators (protons, temperature, and PKC) can increase the extent of partial agonism and attenuate the extent of partial antagonism. Here, we examined the combined effect

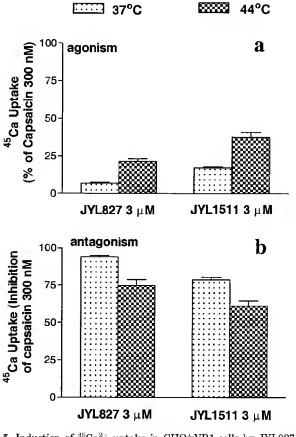


Fig. 5. Induction of $^{45}Ca^{2+}$ uptake in CHO/rVR1 cells by JYL827 and JYL1511 as a function of temperature. a, $^{45}Ca^{2+}$ uptake induced by JYL827 and JYL1511. Data are expressed as percentage of response induced by 300 nM capsaicin after subtraction of the baseline for medium alone. b, $^{45}Ca^{2+}$ uptake induced by JYL827 and JYL1511. Data are expressed as percentage of inhibition of the response induced by 300 nM capsaicin after subtraction of the baseline for medium alone. All values represent mean values of four determinations in three individual, representative experiments; error bars indicate S.E.M.

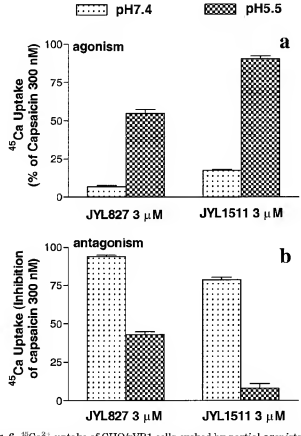


Fig. 6. $^{45}Ca^{2+}$ uptake of CHO/rVR1 cells evoked by partial agonists as a function of pH. a, $^{45}Ca^{2+}$ uptake induced by JYL827 and JYL1511 as a function of pH. Levels of $^{45}Ca^{2+}$ uptake over baseline were expressed relative to that induced by 300 nM capsaicin at the same pH. b, $^{45}Ca^{2+}$ uptake induced by JYL827 and JYL1511 as a function of pH. Data are expressed as percentage of inhibition of the response induced by 300 nM capsaicin after subtraction of the baseline for medium alone. All values represent mean values of four determinations in three individual, representative experiments; error bars indicate S.E.M.

of these three coactivators on the partial agonism/antagonism of JYL827 and JYL1511 (Fig. 8). The three coactivators functioned together to increase the extent of partial agonism of JYL 827 from 6.8 ± 0.7% (n = 7 experiments) to 89.4 ± 2.8% (n = 3 experiments) and that of JYL1511 from 17.4 ± 0.6% (n = 6 experiments) to 98.1 ± 2.6% (n = 3 experiments). Conversely, the combination of coactivators reduced the extent of antagonism of JYL827 from 93.9 ± 0.3% (n = 7 experiments) to 6.3 ± 3.2% (n = 3 experiments) and that of JYL1511 from 84.1 ± 3.2% (n = 6 experiments) to no antagonism. We conclude that protons, high temperature, and PKCs together further enhance the extent of partial agonism and, in the case of JYL827, convert a compound from virtually a complete antagonist (at 22°C, data not shown) to virtually a complete agonist.

Discussion

Partial agonists for VR1 have received relative little attention. Walpole and Wrigglesworth (1993) have described reduced efficacy (20–40%) for *meta*-chloro/fluoro, *p*-hydroxyphenyl derivatives of capsaicin. Likewise, 2-iodo-4-hydroxy-5-methoxy-RTX was reported to display 50 ± 13% of the efficacy of capsaicin (McDonnell et al., 2002), in contrast to 4-hydroxy-5-iodo-3-methoxy RTX, which was a complete antagonist (Wahl et al., 2001). In neither case was it shown that the fractional efficacy arose from partial agonism. This concern is not simply theoretical, because we have observed other compounds that show reduced efficacy without antagonism, presumably reflecting the complexities of the $^{45}Ca^{2+}$ assay (J. Lee, J. Lee, M. Kang, M. Y. Shin, J. M. Kim, S. U. Kang, J. O. Lim, H. K. Choi, Y. G. Suh, H. G. Park, et al., submitted). Finally, anandamide has been described as having partial efficacy for rat (Zygmunt et al., 1999) but not human VR1 (Smart et al., 2000). In the case of anandamide, transporter-dependent uptake and metabolism contribute to its apparent partial efficacy (De Petrocellis et al., 2001).

For JYL827 and JYL1511, we have demonstrated here that their fractional efficacy reflects their functioning as partial agonists with a corresponding degree of partial antagonism. The compounds further provide insights into structure activity relationships. The structure of JYL1511 demonstrates that the 4-methylsulfonylamino group on the A-region by itself does not assure antagonism. Rather, the complete antagonism of compound 2 (*N*-(4-*tert*-butylbenzyl)-*N*′-[4-(methylsulfonylamino)benzyl]thiourea) (Suh et al., 2002a; Wang et al., 2002) is converted into the partial agonism/antagonism of

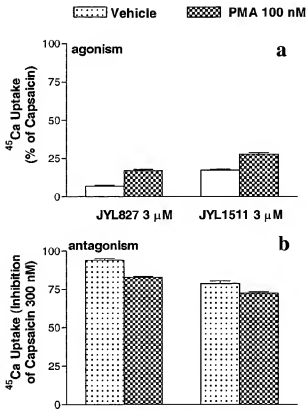


Fig. 7. $^{45}Ca^{2+}$ uptake of CHO/rVR1 cells evoked by partial agonists in the absence or presence of PMA. a, $^{45}Ca^{2+}$ uptake induced by JYL827 and JYL1511 as a function of PMA treatment. Levels of $^{45}Ca^{2+}$ uptake over baseline were expressed relative to that induced by 300 nM capsaicin at the same concentration of PMA. b, $^{45}Ca^{2+}$ uptake induced by JYL827 and JYL1511 as a function of PMA treatment. Data are expressed as percentage of inhibition of the response induced by 300 nM capsaicin after subtraction of the baseline for medium alone. All values represent mean values of four determinations in three individual, representative experiments; error bars indicate S.E.M.

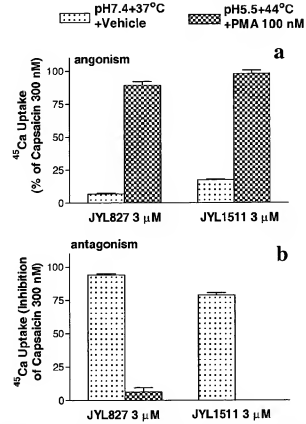


Fig. 8. Effect of the combination of protons, temperature, and PMA on the extent of partial agonism/antagonism. a, $^{45}Ca^{2+}$ uptake induced by JYL827 and JYL1511 as a function of protons, temperature, and PMA. Levels of $^{45}Ca^{2+}$ uptake over baseline were expressed relative to that induced by 300 nM capsaicin under the same conditions. b, $^{45}Ca^{2+}$ uptake induced by JYL827 and JYL1511 as a function of protons, temperature, and PMA. Data are expressed as percentage of inhibition of the response induced by 300 nM capsaicin under the same conditions after subtraction of the baseline for medium alone. All values represent mean values of four determinations in three individual, representative experiments; error bars indicate S.E.M.

JYL1511 by the presence of the additional 3-methoxy group. Furthermore, although the A-region makes a major contribution to the extent of agonism/antagonism, it is clear that the C-region also contributes. Thus, replacement of the *N*-[2-(3,4-dimethylbenzyl)-3-(pivaloyloxy)propyl] moiety in the partial agonist JYL827 with the *N*-(4-*tert*-butylbenzyl) moiety (compound 2) (Suh et al., 2002a; Wang et al., 2002) converted it into a complete antagonist.

A noteworthy feature of the partial agonists described here, differentiating them from the full antagonists for capsaicin action that we described previously (Wang et al., 2002), was that the partial agonists inhibited only the response to capsaicin but not that to pH or temperature. These latter stimuli potentiated the response to the partial agonists. Whether other compounds might show a different pattern of response remains to be determined.

JYL827 and JYL1511 show that it is possible to attain variable degrees of partial agonism. Thus, under our usual assay conditions JYL827 showed less agonism than did JYL1511. Moreover, for both JYL827 and JYL1511, the degree of agonism was a function of the context in which VR1 was found. Consistent with the results in many systems (Kenakin, 1997), the level of receptor expression was an important determinant of the degree of partial agonism. In addition, temperature, pH, and protein kinase C, three well characterized coactivators of rVR1, all served to enhance the extent of agonism. Although the basis for this synergistic enhancement is not yet understood, it cannot be explained simply by a change in ligand binding affinity, because responses at maximally stimulatory concentrations were determined.

Our findings have implications for screening of VR1 antagonists. We have shown how JYL827 could appear as virtually a complete antagonist at 22°C or as an agonist of good efficacy (89.4% efficacy) at pH 5.5, 44°C in the presence of PMA. The situation was similar as a function of VR1 expression level, where JYL827 shifted from no agonism at a low level of VR1 expression to 23% efficacy at a higher level of expression. Therefore, for consistent results in evaluation of antagonists, assay conditions need to be carefully controlled.

Our findings have potential therapeutic implications as well. It has been suggested that a slow rate of uptake of olvanil into the cell may be responsible for the reduced pungency of this compound (Liu et al., 1997), thereby giving olvanil a more favorable therapeutic index. In a similar fashion, it is possible that partial agonists, by providing a more limited influx of calcium, may differentially affect response and desensitization/defunctionalization of sensory neurons.

Moreover, we have shown here that the behavior of partial agonists is dependent on the cellular context in which VR1 is found. An important implication of these results is that partial agonists may behave differently on different subsets of VR1-containing cells, whether they are distinguished by cell type or by environment, and therefore that different partial agonists may be optimal for different conditions. An underlying conceptual problem with VR1 therapeutics is how to achieve a local effect from systemic administration. The modulated behavior of partial agonists may provide one approach. For example, inflammation may be associated with both a locally lowered pH as well as the release of inflammatory mediators such as bradykinin, which can lead to PKC activation. A compound such as JYL827 should preferentially activate VR1 in this environment and might thereby give local desensitization/defunctionalization. Partial agonists such as JYL827 or JYL1511, together with those that may be developed through other synthetic programs, may permit such concepts to be further evaluated.

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Exhibit Y

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Subthreshold concentration of endothelin-1-enhanced, capsaicin-induced bronchoconstriction in anaesthetized guinea-pigs

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Subthreshold concentration of endothelin-1-enhanced, capsaicin-induced bronchoconstriction in anaesthetized guinea-pigs. H. Kanazawa, H. Fujiwara, K. Hirata, J. Yoshikawa. ©ERS Journals Ltd 1998.

ABSTRACT: An increasing number of studies have been performed to address a possible role for endothelin-1 (ET-1) as a significant mediator in asthma. However, the effects of subthreshold concentrations of ET-1, which cannot elicit bronchial smooth muscle contraction itself, in asthma has yet to be determined. This study determined these effects of ET-1 on capsaicin-induced bronchoconstriction in anaesthetized guinea-pigs.

Aerosolized ET-1 administered at doses of 10^{-8} M and higher induced a dose-dependent increase in pulmonary resistance, but ET-1 at 10^{-9} M did not have any bronchoconstrictive effect. However, this subthreshold concentration of ET-1 potentiated capsaicin-induced bronchoconstriction. In addition, the potentiation of capsaicin-induced bronchoconstriction by this subthreshold concentration of ET-1 was completely abolished by BQ788 (ETB receptor antagonist), but not BQ123 (ETA receptor antagonists). Immunoreactive substance P (SP) levels in bronchoalveolar lavage fluid after capsaicin administration were significantly higher than those after solvent administration. However, ET-1 alone did not significantly stimulate immunoreactive SP release and ET-1 (10^{-10} M) did not potentiate capsaicin-induced immunoreactive SP release. In contrast, ET-1 (10^{-9} M) potentiated exogenous neurokinin A- and SP-induced bronchoconstriction.

These findings suggest that a subthreshold concentration of endothelin-1 does not potentiate the tachykinin release induced by capsaicin but the airway smooth muscle contraction through endothelin-B receptors.
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Endothelin-1 (ET-1) is a 21 amino acid peptide originally isolated from porcine endothelial cells [1], which has potent spasmogenic activity in both vascular and airway smooth muscle [1, 2]. Bronchial asthma is an inflammatory airway disorder characterized by bronchoconstriction and bronchial hyperreactivity. Recent observations suggest that an increased intrapulmonary production of ET-1 may specifically occur in asthma. The bronchial epithelium of asthmatic patients has been found to express preproendothelin-1 messenger ribonucleic acid (mRNA) [3], to contain endothelin immunoreactivity [4] and to release large amounts of ET-1 [5]. The high potency of ET-1 in inducing contraction of airway smooth muscle both *in vivo* and *in vitro* [2, 6] led to the assumption that it may play an important role in the pathogenesis of asthma. However, the precise mechanisms through which ET-1 causes bronchoconstriction are unclear. Moreover, it should be stressed that the amount of ET-1 recovered in the bronchoalveolar lavage (BAL) fluid of patients with asthma is quite low compared with the dose of ET-1 found to be effective in eliciting bronchial smooth muscle contraction [7]. It was previously found that a subthreshold concentration of ET-1 which was not able to elicit bronchial smooth muscle contraction markedly augmented the magnitude and duration of bronchoconstriction caused by other mediators, such as histamine [8].

The pulmonary airways of rodents and humans are innervated with sensory C-fibres [9], which provide an afferent pathway for central neural reflex control of airway functions. These neurons also synthesize and store neuropeptides in granules found within their terminal varicosities. A variety of chemical, physical and electrical stimuli can cause sensory C-fibres to release their neuropeptides locally into innervated structures, where these substances often initiate important physiological effects. In the airways, C-fibre stimulation causes the release of two classes of neuropeptides, tachykinins including substance P (SP) and neurokinin A (NKA), and calcitonin-gene related peptide (CGRP) [10]. Capsaicin is thought to stimulate pulmonary and bronchial C-fibre endings directly [11] and has been used as a research tool to identify C-fibre endings. Furthermore, activation of capsaicin-sensitive C-fibres leads to the local release of sensory neuropeptides. It is well known that sensory neuropeptides mimic many of the pathological features of bronchial asthma, including bronchospasm [12], plasma protein extravasation [13], inflammatory cell recruitment [14] and hypersecretion [15] and it has been suggested that overexcitation of sensory fibres

is involved in the pathogenesis of bronchial asthma [16]. This study was designed to determine the physiological roles of subthreshold concentration of ET-1 on capsaicin-induced bronchoconstriction in anaesthetized guinea-pigs.

Materials and methods

Measurement of pulmonary resistance and aerosol generation

Male Hartley guinea-pigs weighing 400–500 g were used. Under sodium pentobarbital anaesthesia (Abbott Laboratories, North Chicago, IL, USA) (50 mg·kg^{-1}, *i.p.*), artificial ventilation was performed through a tracheal cannula connected to a constant-volume ventilator (Model 680; Harvard Apparatus Co., South Natick, MA, USA) at a rate of 60 breaths·min^{-1}. The tidal volume was set at 6 mL·kg^{-1}. Airflow was monitored continuously with a pneumotachograph (TV-241T; Nihon Koden Co., Tokyo, Japan) connected to a differential pressure transducer (TP-602T; Nihon Koden). The tidal volume was calculated by electrical integration of airflow. A fluid-filled polyethylene catheter was introduced into the oesophagus to measure oesophageal pressure as an approximation of pleural pressure. In-tratracheal pressure was measured using a polyethylene catheter inserted into the short tube connecting the tracheal cannula to the pneumotachograph. Transpulmonary pressure (defined as the difference between the intratracheal and the oesophageal pressure) was measured with a differential pressure transducer. Total pulmonary resistance (R_L) was calculated using methods described previously [17]. Before experiments were performed, guinea-pigs were allowed 20 min to recover from the preparation procedure. To prevent alveolar atelectasis, a large inflation of three tidal volumes was performed every 5 min by occluding the expiratory valve. Drug aerosols (mass median aerodynamic diameter 1.8 μM (geometric SD: 2 μM); output 1.5 mL·min^{-1}) were generated by an ultrasonic nebulizer (TUR-3200; Nihon Koden) placed in the inspiratory line of the ventilator. Because capsaicin activates cholinergic reflexes [18], atropine (1 μmol·kg^{-1}) was routinely administered 15 min before drug challenge in all animals.

Effect of endothelin-1 on capsaicin-induced bronchoconstriction

The concentration response of R_L to ET-1 administration was determined using the following method. After a control challenge with the solvent used to prepare ET-1, aerosolized ET-1 was administered (40 breaths at each concentration). R_L was measured 60 s after the end of ET-1 administration, when the bronchoconstrictor response reached its maximum. In subsequent experiments, guinea-pigs were exposed to ET-1 (10^{-10} M, 40 breaths) and 5 min later to capsaicin (2×10^{-6} M or 4×10^{-6} M, 40 breaths at each concentration). The time of exposure to capsaicin was defined as time 0. In another set of experiments, the inhibitory effects of endothelin receptor antagonists on capsaicin-induced bronchoconstriction following pretreatment with ET-1 (10^{-10} M) were evaluated. Guinea-pigs were exposed to BQ123 (10^{-5} M, 40 breaths) or BQ788 (10^{-5} M, 40 breaths) and 5 min later to ET-1 (10^{-10} M, 40 breaths). In following experiments, a specific peptide leukotriene antagonist (ONO-1078, 1 mg·kg^{-1}), thromboxane A_2 (TxA_2) antagonist (S-1452, 0.1 mg·kg^{-1}), histamine antagonist (diphenhydramine, 10 mg·kg^{-1}) and cyclooxygenase inhibitor (indomethacin, 1 mg·kg^{-1}) were given in-travenously. Ten minutes after administration of these agents, guinea-pigs were exposed to ET-1 (10^{-10} M) and 5 min later to capsaicin (2×10^{-6} M) (time o). In a preliminary study, it was determined that administration of BQ788 (10^{-5} M) completely antagonized the contractile activity of ET-1 (10^{-9} or 10^{-8} M).

Measurement of immunoreactive substance P

Immunoreactive SP was measured by the following method, as described previously [19]. Guinea-pigs were exposed to saline or ET-1 (10^{-10} or 10^{-7} M, 40 breaths at each concentration) and 5 min later to capsaicin (4×10^{-6} M, 40 breaths). Guinea-pigs were killed by exsanguination through the carotid artery 2.5 min after capsaicin administration. Saline (5 mL, 37°C) was then instilled into the lung and lavage was repeated three times. Recovered BAL fluid was immediately mixed with 1 mL inhibitor solution (2×10^{-5} M neutral endopeptidase inhibitor phosphoramidon, 500 KIU·mL^{-1} serine protease inhibitor aprotinin and 1.2 mg·mL^{-1} of ethylenediaminetetraacetic acid (EDTA)) to avoid SP degradation and stored at -70°C until assay. Cells were sedimented by centrifugation at 200×*g* for 10 min at 4°C. The supernatant obtained was loaded on reversed-phase C_{18} cartridges (Sep-Pak C_{18}; Millipore, Milford, MA, USA). After washing with 20 mL 4% acetic acid (pH 4.0) and 20 mL distilled water, SP was eluted with 2 mL 80% acetonitrile in 0.1% tri-fluoroacetic acid. Eluates were concentrated by spin-vacuum evaporation, lyophilized, and dissolved with 0.15 mL assay buffer (50 nM phosphate buffer, pH 7.2, containing 3.7 mg·mL^{-1} EDTA and 0.5% bovine serum albumin). A total of 0.1 mL of the dissolved preparation was subjected to further radioimmunoassay (RIA) for SP. RIA for SP was performed using ^{125}I-labelled SP (Amersham International, Amersham, UK) and anti-SP rabbit serum (Amersham International). The possible cross-reactivity of the SP-antibody with other tachykinins was 1% for NKA. A total of 0.1 mL sample was mixed with 0.5 mL assay buffer, 0.1 mL antiserum, and 0.1 mL ^{125}I-SP and stored at 4°C for 24 h. A 0.2 mL suspension of dextran, charcoal (0.2% dextran and 2% activated charcoal in assay buffer) was added to the reaction mixture and centrifuged at 200×*g* for 10 min. The radioactivity of the supernatant was measured by a gamma counter (Auto-Gamma 5550, Packard Instrument Co, Chicago, IL, USA). In this system, the sensitivity of immunoassayable SP in saline was 1–150 fmol·mL^{-1}. Using this protocol, the recovery of added radiolabelled SP was 85–90%.

Effect of endothelin-1 on substance P- and neurokinin A-induced bronchoconstriction

Guinea-pigs were exposed to ET-1 (10^{-10} M) and 5 min later to NKA (5×10^{-6} M, 40 breaths) or SP (5×10^{-6} M, 40

breaths). The effect of ET-1 was evaluated 5 min after NKA or SP administration. In the following experiments, the inhibitory effects of BQ788 on exogenous tachykinin-induced bronchoconstriction following pretreatment with ET-1 were also evaluated.

Effect of endothelin-1 on acetylcholine-induced bronchoconstriction

Guinea-pigs were exposed to ET-1 (10^{-10} M) and 5 min later to acetylcholine (1×10^{-3}, 3×10^{-3} and 5×10^{-3} M; 40 breaths at each concentration). The effect of ET-1 was evaluated 5 min after acetylcholine administration.

Drugs

Capsaicin, diphenhydramine and indomethacin were obtained from Sigma Chemical Co. (St Louis, MO, USA). NKA, SP and ET-1 were purchased from Peptide Institute (Osaka, Japan). Cyclo (D-Trp-D-Asp-L-Pro-D-Val-L-Leu) (BQ123) and N-cis-2, 6-dimethylpiperidinocarbonyl-L-γ-methyleucyl-D-methoxycarbonyltryptophanyl-D-norleucine (BQ788) were purchased from RBI (Natick, MA, USA). BQ123 and BQ788 were dissolved in ethanol and further dilutions were performed in 0.9% saline. 4-OXO-8-[*N*-(4-phenylbutyloxy) benzoylamino]-2-(tetrazol-5-yle) -4H-1-benzopyran hemihydrate (ONO-1078) and calcium 5 (Z)-1R, 2S, 3S, 4S-7-[3 phenylsulphonylaminobicyclo [2, 2, 1] hept-2-yl]-5-heptenoate hydrate (S-1452) were kindly provided by ONO Pharmaceutical Co. (Osaka, Japan) and Shionogi Pharmaceutical, Osaka, Japan), respectively.

Statistical analysis

All values are expressed as mean±SEM. The statistical significance was determined by analysis of variance (ANOVA); $p<0.05$ was considered significant. When ANOVA disclosed a significant difference, the Newman–Keuls test was used to determine which groups were significantly different from each other.

Results

The baseline *RL* after administration of solvent alone was 0.18±0.02 $cmH_2O{\cdot}mL^{-1}{\cdot}s^{-1}$ and acrosolized ET-1 administration resulted in a dose-dependent increase in *RL* (fig. 1). However, ET-1 (10^{-10} M) did not have any bronchoconstrictive effect.

Acrosolized capsaicin (2×10^{-6} M) administration did not significantly increase *RL* compared with solvent alone, but this concentration of capsaicin significantly increased *RL* following pretreatment with a subthreshold concentration of ET-1 (10^{-10} M) (table 1). In addition, capsaicin (4×10^{-6} M) significantly increased *RL* compared with solvent alone, and a subthreshold concentration of ET-1 markedly potentiated capsaicin (4×10^{-6} M)-induced bronchoconstriction. The solvent used for BQ123 and BQ788 had no effect

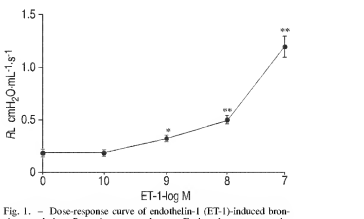

Fig. 1. – Dose-response curve of endothelin-1 (ET-1)-induced bronchoconstriction. *RL*: pulmonary resistance. Each point represents the mean±SEM for six animals. *: $p<0.05$, **: $p<0.01$ compared with solvent alone.

on capsaicin-induced bronchoconstriction. BQ788, but not BQ123, significantly inhibited capsaicin (4×10^{-6} M)-induced bronchoconstriction following pretreatment with a subthreshold concentration of ET-1 (fig. 2). However, receptor antagonists of leukotriene, thromboxane A_2, and histamine had no significant effects on the potentiation by ET-1 of capsaicin-induced bronchoconstriction. Moreover, the cyclooxygenase inhibitor had no effect on this potentiation by ET-1 (table 2).

Immunoreactive SP levels in BAL fluid after capsaicin administration were significantly higher than those after solvent administration (48±7 $fmol{\cdot}L^{-1}$ for solvent and 120±20 $fmol{\cdot}L^{-1}$ for 4×10^{-6} M capsaicin; $p<0.01$) (fig. 3). ET-1 (10^{-10} or 10^{-7} M) alone did not induce significant release of immunoreactive SP. Moreover, ET-1 (10^{-10} M) did not potentiate capsaicin-induced immunoreactive SP release. NKA (5×10^{-6} M) and SP (5×10^{-6} M) each induced significant increases in *RL* (0.45±0.05 and 0.35±0.05 $cmH_2O{\cdot}mL^{-1}{\cdot}s$, respectively) (fig. 4). ET-1 (10^{-10} M) significantly potentiated exogenous NKA- and SP-induced bronchoconstriction. Although the solvent used for BQ788 had no effect on tachykinin-induced bronchoconstriction, BQ 788 (10^{-5} M) could completely inhibit the potentiation by ET-1 (10^{-10} M) of NKA or SP-induced bronchoconstriction. More-

Table 1. – Effect of subthreshold concentration of endothelin-1 (ET-1) on capsaicin-induced bronchoconstriction

	RL $cmH_2O{\cdot}mL^{-1}{\cdot}s$			
	2.5 min	5 min	7.5 min	10 min
Solvent	0.19±0.02	0.19±0.02	0.18±0.02	0.18±0.02
Capsaicin (2×10^{-6} M)	0.27±0.05	0.25±0.04	0.22±0.02	0.20±0.02
Capsaicin (4×10^{-6} M)	0.74±0.11††	0.61±0.09††	0.42±0.07††	0.34±0.05†
ET-1 (10^{-10} M) + Capsaicin (2×10^{-6} M)	0.41±0.07*	0.40±0.06*	0.38±0.04*	0.36±0.03*
+ Capsaicin (4×10^{-6} M)	1.27±0.21‡	0.91±0.11‡	0.81±0.10‡	0.74±0.08‡

Each value represents the mean±SEM for six animals. *RL*: pulmonary resistance. †: $p<0.05$; ††: $p<0.01$, compared with solvent alone; *: $p<0.05$ compared with capsaicin (2×10^{-6} M) alone; ‡: $p<0.01$ compared with capsaicin (4×10^{-6} M) alone.

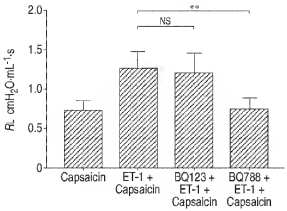

Fig. 2. – Inhibitory effects of endothelin (ET)A (BQ123) or ETB (BQ-788) receptor antagonists (both 10^{-5} M) on capsaicin-induced (4×10^{-6} M) bronchoconstriction following pretreatment with ET-1 (10^{-10} M). *RL*: pulmonary resistance. Each column corresponds to 2.5 min after capsaicin administration and represents the mean±SEM for six animals. **: p<0.01.

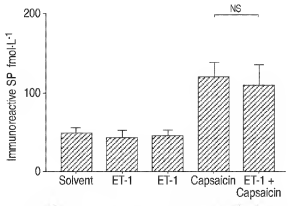

Fig. 3. – Effects of endothelin-1 (ET-1; 10^{-10} M) on capsaicin-induced (4×10^{-6} M) immunoreactive substance P (SP) levels in bronchoalveolar lavage fluid. Each column corresponds to 2.5 min after capsaicin administration and represents the mean±SEM for seven animals. The immunoreactive SP level after administration of capsaicin was significantly higher than that after solvent alone (p<0.01).

over, ET-1 (10^{-10} M) significantly potentiated acetylcholine-induced bronchoconstriction (fig. 5).

Discussion

Activation of airway C-fibres by capsaicin causes the release of neuropeptides, resulting in bronchoconstriction. In this study, a subthreshold concentration of ET-1 was found to potentiate capsaicin-induced bronchoconstriction. This subthreshold concentration of ET-1 did not potentiate the release of SP immunoreactivity stimulated by capsaicin and exogenous tachykinin administration markedly enhanced bronchoconstriction following pretreatment with a subthreshold concentration of ET-1. These findings suggest that although a subthreshold concentration of ET-1 does not stimulate tachykinin release from capsaicin-sensitive nerve endings, this dose of ET-1 enhances tachykinin-induced bronchoconstriction at postjunctional levels.

Previous studies have revealed potential mechanisms *via* which ET-1 may induce bronchoconstriction either through a direct effect on airway smooth muscle; or through an indirect effect secondary to mediator release. ET-1 increases intracellular calcium and activates phospholipase C, generating inositol-triphosphate and diacylglycerol in human bronchial smooth muscle cells [20]. In guinea-pig airways, partial inhibition of ET-induced contraction can be obtained by preincubation with nifedipine [21]. Tachykinins released by capsaicin possibly induce the increase in intracellular Ca^{2+} levels in airway smooth muscle cells. Consequently, ET-1 and tachykinins would concomitantly stimulate the influx of Ca^{2+} into airway smooth muscle cells and Ca^{2+} release from sarcoplasmic reticulum, and enhance the contraction of airway smooth muscle. In guinea-pig trachea, TxA_2, platelet-activating factor (PAF), leukotriene and adenosine have been suggested as secondary mediators of ET-1-induced bronchoconstriction [22, 23]. However, TxA_2, PAF, leukotriene and histamine receptor antagonists did not affect ET-1-induced contraction in human isolated bronchi [24].

In this study, receptor antagonists of leukotriene, TxA_2 and histamine, and cyclooxygenase inhibitor were shown to have no significant effects on the potentiation by a low dose of ET-1 of capsaicin-induced bronchoconstriction. These findings suggest that a low dose of ET-1 may affect guinea-pig bronchi predominantly through a direct effect.

Table 2. – Effect of receptor antagonists of leukotriene, thromboxane A_2 and histamine and cyclooxygenase inhibitor on the potentiation by endothelin-1 (ET-1) of capsaicin-induced bronchoconstriction

	RL cmH$_2$O·mL^{-1}·s			
	2.5 min	5 min	7.5 min	10 min
ONO-1078 (1 mg·kg^{-1})	0.36±0.08	0.39±0.07	0.40±0.06	0.40±0.05
S-1452 (0.1 mg·kg^{-1})	0.38±0.07	0.40±0.04	0.40±0.04	0.40±0.04
Diphenhydramine (10 mg·kg^{-1})	0.41±0.06	0.42±0.05	0.41±0.05	0.41±0.05
Indomethacin (1 mg·kg^{-1})	0.39±0.07	0.39±0.07	0.40±0.07	0.42±0.06

Each value represents the mean±SEM for five animals. *RL*: pulmonary resistance. ONO-1078, S-1452, diphenhydramine and indomethacin had no significant effects on the potentiation by ET-1 of capsaicin (2×10^{-6} M)-induced bronchoconstriction.

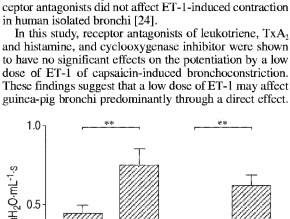

Fig. 4. – Effects of endothelin-1 (ET-1; 10^{-10} M) on neurokinin A (NKA; 5×10^{-4} M) and substance P (SP)-induced bronchoconstriction (5×10^{-4} M). *RL*: pulmonary resistance. Each column shows results obtained at 2.5 min after tachykinin administration and represents the mean±SEM for six animals. **: p<0.01.

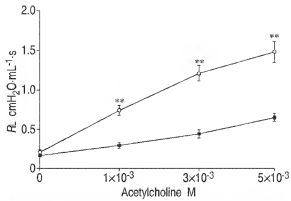

Fig. 5. – Effects of endothelin-1 (ET-1; 10^{-10} M) on acetylcholine-induced bronchoconstriction. ●: solvent; ○: ET-1. Each point represents the mean±SD for five animals. **: $p<0.01$ compared with acetylcholine alone.

However, ET-1 (10^{-10} M) also potentiated acetylcholine-induced bronchoconstriction. Accordingly, a subthreshold concentration of ET-1 may cause nonspecific bronchial hyperresponsiveness. Although acetylcholine is thought to be a direct smooth muscle constrictor, previous re- ports suggested that the administration of methacholine into guinea-pig airways induced tachykinin release [25]. Further studies will be required to determine whether a subthreshold concentration of ET-1 induces nonspecific airway hyperresponsiveness. A further, third mechanism of ET-1-induced bronchoconstriction is mediated through a neuromodulator effect that potentiates neurally mediated bronchoconstriction [26].

Various effects of ET-1 are mediated *via* ET receptor, and two subtypes of ET receptor, termed ETA and ETB, have been cloned, sequenced and characterized [27, 28]. In guinea-pig and isolated human bronchial smooth muscle, ET-1-induced contraction is mediated predominantly *via* activation of the ETB receptor subtype [29, 30]. However, contractions in response to ET-1 in the guinea-pig trachea and lung parenchyma are mediated in part by ETA receptors. In addition, in lung parenchyma, these ETA receptors mediate contraction through the release of cyclooxygenase metabolites induced by relatively high doses of ET-1. In this study, potentiation of capsaicin-induced and tachykinin-induced bronchoconstriction by subthreshold concentration of ET-1 was completely abolished by BQ788, suggesting that low concentrations of ET-1 affect airway responses through ETB receptors alone.

ET-1 acts primarily as a local paracrine and autocrine hormone. Even though ET-1 concentrations in BAL fluid are generally below the threshold level required for contraction of airway smooth muscles, ET-1 levels in local airways are possibly high enough to elicit bronchoconstriction [31]. However, it is also important to determine the physiological effects of a subthreshold concentration of ET-1 acting as a local hormone. A recent study determined that inhaled ET-1 is a potent bronchoconstrictor, with a bronchoconstrictive potency about 100-times that of methacholine in asthmatic patients [32]. Furthermore, it remains to be determined whether or not there are therapeutic advantages in using selective ET receptor antagonists in the treatment of asthma. Further studies will be needed to elucidate and classify the ET receptor subtypes mediating the physiological effects of ET-1 in airway responses.

In conclusion, this study suggests that a subthreshold concentration of endothelin-1 potentiated airway smooth muscle contraction through endothelin-B receptors. The effect was not mediated through enhanced tachykinin release from capsaicin-sensitive nerve endings, but occurred at a postjunctional level.

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Exhibit Z

ELSEVIER

Airway sensory innervation as a target for novel therapies: an outdated concept?

Maria G Belvisi

Sensory nerves in the airways regulate central and local reflex events such as bronchoconstriction, airway plasma leakage, mucus secretion and cough. Sensory nerve activity can be enhanced during inflammation and, as a result, these protective reflexes become exacerbated and deleterious. The development of drugs that directly inhibit sensory nerve function has again become an attractive target for the pharmaceutical industry. In particular, the focus is on inhibition of the symptoms associated with airway inflammatory diseases such as asthma, chronic obstructive pulmonary disease and cough of any aetiology.

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Abbreviations

BK_{Ca}	large conductance calcium-activated potassium (channel)
COPD	chronic obstructive pulmonary disease
NANC	non-adrenergic non-cholinergic
NK	neurokinin
RAR	rapidly adapting stretch receptor
SP	substance P
TRP	transient receptor potential

Introduction

Sensory nerves in the airways regulate central and local reflex events such as bronchoconstriction, airway plasma leakage and cough [1••]. During inflammation, sensory nerve activity can become enhanced, resulting in these protective reflexes become exacerbated and deleterious [1••]. Sensory nerve reflexes are under the control of two different classes of sensory fibre: myelinated, rapidly adapting stretch receptors (RARs) and non-myelinated, capsaicin-sensitive C-fibres [2,3]. In the airways, activation of RARs and C-fibres elicits cough, bronchoconstriction and mucus secretion through an afferent central reflex pathway [4–6]. Activation of C-fibres also mediates efferent excitatory, non-adrenergic non-cholinergic (NANC) responses, such as bronchoconstriction, mucus secretion, plasma exudation and vasodilatation, through the peripheral release of neuropeptides, a phenomenon known as 'neurogenic inflammation' [1••].

Current drugs (β-adrenoceptor agonists, theophylline and anti-cholinergics) do not alleviate all symptoms (e.g. cough and neurogenic inflammation) associated with asthma and chronic obstructive pulmonary disease (COPD). This review focuses on compounds that target receptors, ion channels or other molecules expressed peripherally on sensory nerve terminals, and discusses their utility in treating the symptomatology of airway inflammatory diseases. Furthermore, we hypothesise that compounds which directly inhibit sensory nerve activity, resulting in a reduction of both afferent and efferent function, could inhibit the cough, neurogenic inflammation and central reflexes that result in parasympathetic cholinergic bronchoconstriction and mucus secretion, thus providing a more global therapy and comprehensive symptom management regimen.

Sensory innervation of the airways

The vagus nerves supply most of the sensory nerve fibres to the airways. Some sensory innervation originates from the dorsal root ganglia, with fibres running together with spinal sympathetic nerves. The cell bodies of vagal sensory fibres are located in the jugular and nodose ganglia, with peripheral and central projections to the airways and medulla, respectively. As stated previously, sensory nerve reflexes are controlled by RARs and C-fibres [2,3]. Interestingly, activation of C-fibres in the airways also mediates efferent excitatory NANC responses known as 'neurogenic inflammation' [1••].

Inappropriate activation of these nerves leads to many of the symptoms of allergic diseases (e.g. asthma) and COPD, such as coughing, mucus hypersecretion and bronchoconstriction [1••]. The mechanisms involved in the abnormal functioning of airway nerves in inflammatory disease have not been described. However, they are thought to involve the release of mediators that sensitise nerve fibres, leading to increased electrical activity of these fibres and an increase in the release of neurotransmitters from the nerve endings [6].

Role of airway sensory nerves in central reflex events

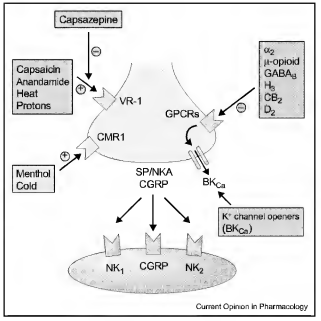
RARs respond readily to changes in the mechanical properties of the lungs and airways, and are of functional importance in airway defence, as their stimulation has been shown to elicit a variety of defensive reflexes

including cough. A characteristic feature of C-fibres is their chemosensitivity and, in particular, their sensitivity to capsaicin, the pungent principal in peppers of the *Capsicum* family. Information regarding the cellular specificity of capsaicin in the airways has been obtained from *in vitro* and *in vivo* single fibre recordings. Fox, Barnes, Urban and Dray [7] found that, in the guinea-pig, capsaicin was a highly selective stimulant of C-fibres, producing no firing of myelinated Aδ-fibres (corresponding to RARs). However, using a similar *in vitro* preparation, others have recently described a sub-population of Aδ-fibres that are sensitive to capsaicin [8]. *In vivo* studies using cats have shown that a proportion of RARs can be excited by capsaicin, although higher concentrations are needed than those required to activate C-fibres [9,10]. It has recently been determined that the actions of capsaicin on sensory nerves are mediated through the stimulation of a specific membrane-bound receptor, now known to be a ligand-gated ion channel termed the VR-1 receptor [11–13] A VR-1 receptor antagonist, capsazepine, inhibits the cough induced by capsaicin and citric acid in experimental animals [14]. C-fibre endings have also been found to be chemosensitive to several other agents, including prostanoids, ATP, bradykinin, hypertonic saline, low-chloride solutions, cigarette smoke and ozone. However, the mechanism by which these agents activate C-fibres is not known. Unlike the RARs, excitation of C-fibres by these agents appears to be direct and can be elicited at concentrations that do not cause other effects, such as bronchoconstriction.

Role of C-fibres in local reflexes: neurogenic inflammation

The population of sensory nerves known as the chemosensitive C-fibres are thought to contain neuropeptides, particularly calcitonin gene-related peptide and the tachykinins substance P (SP) and neurokinin (NK)A. The stimulation of these nerve endings can result in a local or axon reflex, causing the release of sensory neuropeptides contained within; this is the so-called 'efferent' function of sensory nerves [15] (Figure 1). SP and NKA are potent inducers of airway smooth muscle contraction, vasodilatation, bronchial oedema and mucus hypersecretion, which are all classic symptoms of inflammatory airway diseases. Therefore, neuropeptides released from nerve endings through such a reflex have been proposed to be important in the pathology of diseases such as asthma [5,15,16]. It has been demonstrated that electrically evoked bronchoconstriction *in vitro* and *in vivo* is blocked by NK_1 and NK_2 receptor antagonists [17–19]. Similarly, mucus secretion and microvascular leakage, which are seen in the presence of muscarinic and adrenergic receptor blockade, are inhibited by NK_1 receptor antagonists [17,20,21]. Interestingly, NK receptor antagonists have also been shown to be effective anti-tussive agents in animal models, although it is unclear if this is caused by a central or peripheral action [22]. The NK_2 receptor antagonist SR 48968 has been shown to inhibit citric acid-induced cough in conscious guinea-pigs [23,24]. Recent data have implicated a role for NK_3 receptor activation in evoking a tussive response, [25,26] and SB 235375, which is a high affinity (low central nervous system penetrant), selective, reversible and competitive NK_3 receptor antagonist, is also effective against citric acid-induced cough in guinea-pigs [26]. Consistent with these data, a recent paper using a novel $NK_1/NK_2/NK_3$ receptor antagonist, SCH 206272, showed inhibition of capsaicin-induced cough in the same model [27].

Figure 1



Numerous studies have shown that NANC bronchoconstriction in guinea-pigs can be inhibited by activation of μ-opioid receptors, $GABA_B$ receptors, α_2-adrenoceptors and neuropeptide Y receptors, implying inhibition of neuropeptide release from sensory nerve endings. Much less information is available on whether the afferent activity of sensory nerves can also be inhibited. It has been proposed that the inhibitory effect of these receptors may be mediated through a common mechanism of BK_{Ca} opening [39,47]. In addition, the heat-sensitive VR-1 receptor is a TRP channel expressed on C-fibres that can be activated by capsaicin [12,13]. A VR-1 receptor antagonist, capsazepine, inhibits cough in experimental animals [14], suggesting that blockade of this channel may be a good target for anti-tussive therapy. The cold- and menthol-sensitive receptor (CMR1) is also a member of the TRP family of excitatory ion channels that is expressed by a subpopulation of C-fibres (and possibly Aδ-fibres) [48••]. Interestingly, menthol has been proposed as an anti-tussive therapy [49], and so activators of this channel could be effective antitussives. CGRP, calcitonin gene-regulated protein; GPCR, G-protein-coupled receptor.

Strategies for inhibiting sensory nerve-mediated responses

Inhibition of neurogenic inflammation and neurokinin receptor antagonists

The relevance of neurogenic inflammation in human airways is not known. Immunohistochemical studies have

demonstrated the presence of SP-containing nerve fibres in human airways, and the number and length of these fibres were found to be increased in the airways of asthmatics compared with normal controls [28]. However, other studies have failed to reproduce these findings. Although, NANC contractile responses are functionally weak in human airways *in vitro* [29], capsaicin-evoked mucus secretion has been demonstrated from isolated human airways [30]. These weak NANC responses, together with the inconsistent peptide staining, might be caused by degradation of peptides seen in denervated postmortem tissue. Although it is possible that such local reflexes occur in human lower airways, available data argue against this hypothesis. For example, NK receptor antagonists have no effect on capsaicin-induced contractions of human airway preparations [31].

Recently, evidence was provided for *in vivo* NANC responses in humans by the demonstration that bradykinin-induced bronchoconstriction was blocked by a mixed NK_1/NK_2 receptor antagonist [32]. However, subsequent studies have shown that this antagonist does not prevent NKA-induced bronchoconstriction in asthmatics [33]. NK_1 and NK_2 receptor antagonists that are potent, non-peptidic, safe and selective have only recently become available for studies in humans. NKA-induced bronchospasm is inhibited by a selective NK_2 receptor antagonist (SR 48968C) [34]. However, when the same compound was administered orally for nine days in allergic asthmatic patients, it did not have a significant bronchodilatory effect or a bronchoprotective effect against AMP hyperresponsiveness [35]. Further studies into the effects of these selective antagonists in asthma (and other diseases such as COPD) and, more specifically, their effects on parameters such as allergen-induced hyperresponsiveness and subjective asthma symptoms (e.g. chest tightness and cough) should be performed before definitive conclusions are drawn on the role of tachykinins in asthma. Furthermore, the contribution of different NK receptor subtypes is unknown, and it might be that studies using a combined NK_1/NK_2 or even a $NK_1/NK_2/NK_3$ receptor antagonist have to be carried out to determine whether these receptors play a role in asthma. The activity of such molecules in animal models has led to the suggestion that data generated from these systems may not be predictive of the situation in humans. Indeed, the lack of evidence for clinical efficacy of this class of compound has led to scepticism within the field regarding the role of sensory nerves in airway inflammatory diseases. However, it should be noted that peripherally acting NK receptor antagonists might not inhibit airway effects resulting from central sensory nerve reflexes.

Prejunctional inhibition of sensory nerve function

Direct modulation of sensory nerve activity could conceivably inhibit airway effects resulting from both central and peripheral reflexes, and may have advantages over other targets (e.g. NK receptor antagonists). Several studies have shown that NANC bronchoconstriction, plasma leakage and mucus secretion can be inhibited by activation of μ-opioid receptors, $GABA_B$ receptors, α_2-adrenoceptors and neuropeptide Y receptors, implying inhibition of neuropeptide release from C-fibre nerve endings [36–41]. However, much less information is available on whether afferent activity of C- and/or Aδ-fibres can also be inhibited.

Several other potential targets have been described that could lead to the development of agents effective at inhibiting central and local sensory nerve reflexes. Interestingly, recent data suggest that cannabinoid CB_2 receptor agonists show anti-tussive activity in animal models [42]. Furthermore, Astra Zeneca have developed a compound called Viozan, which was a first in class dual β_2-adrenoceptor and dopamine D_2 receptor agonist [43]. It was thought that this combination would provide both bronchodilation (through β_2-adrenoceptor activation) and inhibition of sensory nerve function (through D_2 receptor activation). Indeed, dopamine was shown to inhibit histamine-induced discharge of RARs in dog lung. This effect was blocked by the D_2 receptor antagonist sulpiride, providing pre-clinical validation of the hypothesis [44]. However, although this agent could partially block vagal sensory nerve depolarisation evoked by capsaicin *in vitro*, this effect did not appear to be caused by D_2 receptor activation [45]. Furthermore, although Viozan inhibited capsaicin-induced plasma extravasation, this effect was inhibited by the β_2-adrenoceptor antagonist propranolol, suggesting that this novel dual therapy had no advantage over existing β_2-adrenoceptor agonist therapy [46]. Phase II clinical studies in COPD patients have shown that Viozan significantly improves symptoms of cough, breathlessness, sputum production and health status quality of life [43]. Subsequently, this compound has been dropped from clinical development, possibly because of the difficulty in validating the 'proof of concept' for the D_2 agonist part of the molecule in humans.

Interestingly, it has been proposed that the inhibitory effect of several G-protein-coupled receptors may be mediated through a common mechanism by the opening of large conductance calcium-activated potassium (BK_{Ca}) channels [39,41,47] (Figure 1). Activation of BK_{Ca} channels is thought to hyperpolarise sensory nerves, and thus reduce their excitability. A study utilising a novel BK_{Ca} channel opener, NS1619, has demonstrated inhibition both of NANC bronchoconstriction and of single C-fibre firing evoked by bradykinin in guinea-pig airways [47]. NS1619 also inhibited Aδ-fibre firing evoked by distilled water; iberiotoxin, which selectively blocks the BK_{Ca} channel, inhibited all of the actions of NS1619. Furthermore, NS1619 inhibited neurogenic mucus secretion in ferret trachea *in vitro* [41]. Importantly, this compound also inhibited sensory nerve reflexes (i.e. citric acid-induced cough) *in vivo* in the guinea-pig [44].

Conclusions

The lack of clinical activity of NK receptor antagonists has cast some doubt over targeting sensory nerves in the treatment of airway inflammatory diseases. Peripherally acting NK receptors will not address the central reflexes evoked by sensory nerve activation, even though they do inhibit neurogenic inflammation. Several agents have been identified that can directly inhibit sensory nerve activity peripherally, thereby suppressing local and central reflexes (e.g. VR-1 receptor antagonists). Recently, the cold- and menthol-sensitive receptor has been cloned, and is a member of the transient receptor potential (TRP) family of excitatory ion channels that also includes the VR-1 receptor [48**]. The cold- and menthol-sensitive receptor is also expressed on C-fibres. Interestingly, menthol (an agonist at this receptor) has been proposed as an anti-cussive therapy [49], and so activators of this channel could also be effective anti-tussive therapies. However, a more attractive option is to inhibit central and local reflexes by a more generalised action through inhibition of both myelinated and unmyelinated fibres (e.g. via cannabinoid CB_2 receptors and BK_{Ca} channel openers). Currently, there are no effective treatments for certain symptoms of airway inflammatory diseases, such as the cough reflex, with an acceptable therapeutic ratio. However, the future looks promising, with several novel mechanisms identified that could lead to the identification of drugs that target the increased activity of sensory nerves in these diseases, challenging the notion that targeting sensory nerves is an outdated concept.

Acknowledgements

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Sensory nerves and airway inflammation: role of Aδ and C-fibres

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Abstract

It is generally accepted that stimulation of primary afferent sensory neurons, that innervate the airways, by chemical and mechanical stimuli leads to a range of homeostatic and defensive reflexes such as cough. However, there is still much debate regarding the exact type of sensory fibre involved in evoking these reflex events. The current dogma suggests that the major fibre types implicated in participating in reflex events of a protective nature are the Aδ fibres and those stimulated in response to inflammation by noxious stimuli and mediators associated with tissue damage are the unmyelinated C-fibres. Furthermore, the C-fibre afferents are also believed to be responsible for mediating local axon reflexes, the release of neuropeptides and neurogenic inflammation. This review will concentrate on describing the characteristics of these sensory fibres and their proposed role in airway defensive reflexes and their possible exaggerated function in response to the inflammatory process.

Keywords: Sensory nerves; C-fibres; Aδ fibres; Neurogenic inflammation; Neuropeptides

1. Introduction

The vagus nerves supply most of the sensory nerve fibres to the airways. Some sensory innervation originates from the dorsal root ganglia, with fibres running with spinal sympathetic nerves. The vagus divides into the superior laryngeal and recurrent laryngeal nerves, which carry sensory fibres and pre-ganglionic parasympathetic fibres to the trachea and main bronchi. Smaller branches of the vagus then supply the rest of the airways [1–3]. The cell bodies of vagal sensory fibres are located in the jugular and nodose ganglia with projections peripherally to the airways and centrally to the medulla. Inappropriate activation of these nerves can lead to many of the symptoms of allergic diseases (e.g. asthma) and chronic obstructive pulmonary disease (COPD) such as coughing, mucus hypersecretion and bronchoconstriction [4,5]. Similarly, inappropriate activation of trigeminal afferent nerves, comparable to the vagal afferent nerves innervating the lower airways, lead to many of the symptoms associated with allergic rhinitis (including sneezing, mucus secretion, vascular engorgement, plasma exudation). The mechanisms involved in the abnormal functioning of airway nerves in inflammatory disease have not yet been described. However, they are thought to involve the release of mediators which sensitise the nerve fibres leading to increased electrical activity of these fibres and an increase in the release of various neurotransmitters from the nerve endings [6].

The purpose of airway sensory nerves is to relay sensory information to the central nervous system so that appropriate changes in the motor outputs may occur. The effects of stimulation of sensory nerves include the aspiration reflex via stimulation of nerves in the nasopharynx [7], and various respiratory defence reflexes such as apnoea, changes in blood pressure, lower airway mucosal secretion, bronchoconstriction and cough [8]. Sensory nerve endings in the airways form specific receptors which have been classified into different types based on their functional properties. This classification derives from single fibre recording studies, performed in vivo in anaesthetised animals. More recently, the properties of these sensory neurones have been examined in vitro using intracellular recording techniques on isolated nodose ganglia [9], or novel preparations of the isolated trachea and main bronchi, with attached vagus nerve and ganglia [10,11]. These single fibre recording studies have described three classes of afferent fibre ending: slowly adapting stretch receptors, rapidly adapting stretch receptors and C-fibre endings.

2. Physiology of rapidly adapting receptors (RARS)

RARs (also known as irritant receptors) generally have slower conduction velocities, than slowly adapting receptors,

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within the myelinated Aδ-fibre range [8]. They exhibit bursts of irregular activity during a maintained inflation of the lung which rapidly 'adapts' to levels close to the pre-inflation value. Electrophysiological studies have examined their distribution throughout the airways. Whilst they have been found at all airway levels, their concentration is greatest in the larger airways, especially around the carina, hilum and main bronchi, and decreases towards the smaller airways [12]. This localisation here underlies their defensive role as here they are accessible to and may be activated by various chemical and mechanical irritants. They are distributed throughout the airway wall and are highly sensitive to stimulation by light touch indicating a superficial localisation in the epithelium [12,13]. However, the responsiveness of RARs to light touch may not necessarily indicate a superficial localisation. For example, there is anatomical evidence that RARs in guinea-pig airways are located below the epithelium [14].

As a group, RAR's respond readily to changes in the mechanical properties of the lungs and airways and are of functional importance in airway defence, as their stimulation has been shown to elicit a variety of defensive reflexes including cough. However, RAR's are sensitive to a wide range of stimuli including pathophysiological conditions as well as exogenously administered substances.

3. Physiology of C-fibre afferents

Another class of airway sensory fibre is the unmyelinated C-fibre. These may be classified as 'bronchial' or 'pulmonary', based on their accessibility to chemicals injected into either the bronchial or pulmonary arteries. Pulmonary C-fibre endings are located in the lung parenchyma, whilst bronchial C-fibres are located within the airway mucosa, and are predominantly stimulated by agents deposited on this site [8,15]. Any structural differences between the two cannot as yet be stated since neither has been clearly identified histologically.

Pulmonary C-fibre endings are sensitive to increases in lung volume, whilst bronchial C-fibres are less sensitive and unpredictable in their response to inflation. The stimulation of pulmonary C-fibre receptors results in a pulmonary chemoreflex involving bradycardia, hypotension and apnoea followed by rapid shallow breathing [7,8,16]. Bronchial C-fibre stimulation evokes similar responses including bronchoconstriction, rapid shallow breathing, extravasation of plasma and mucus hypersecretion, plus the cough reflex.

A characteristic feature of C-fibres is their chemosensitivity and in particular, their sensitivity to capsaicin, the pungent principal in peppers of the *Capsicum* family. This agent has well established excitatory actions on a sub-population of primary afferent neurones in the skin, joints and viscera of a number of vertebrate species, excluding birds, consisting of neuropeptide-containing C-fibres and some small-diameter Aδ-fibres [17]. Information regarding the cellular specificity of capsaicin in the airways has been obtained from in vitro and in vivo single fibre recordings. In the guinea-pig, Fox et al. [11] found that capsaicin was a highly selective stimulant of C-fibres, producing no firing of myelinated Aδ-fibres (corresponding to RARs). However, it should be noted that more recently, using a similar in vitro preparation, others have described a sub-population of Aδ fibres apparently sensitive to capsaicin [10]. In vivo studies in cats have found that a proportion of RARs may be excited by capsaicin, although at higher concentrations than those required to activate C-fibres [18,19]. It has recently been determined that the actions of capsaicin on sensory nerves are mediated via the stimulation of a specific membrane-bound receptor, now known to be a ligand-gated ion channel [20–22].

C-fibre endings have been found to be chemosensitive to a number of other agents including, bradykinin, hypertonic saline, low-chloride solutions, cigarette smoke and ozone. Unlike the RARs, the excitation of C-fibres by these agents appears to be direct and may be elicited at concentrations which do not have other effects such as bronchoconstriction. However, there are several species variations in the agents that are able to directly activate C-fibres. Prostanoids markedly potentiate responses of C-fibres to subsequent stimulation and markedly potentiate capsaicin-induced cough, but do not appear to directly activate C-fibres in rats or guinea-pigs as they do in dogs and cats. Conversely, it has been reported that adenosine activates C-fibres in rats but not in dogs. There also appear to be differences in the ability of mediators to stimulate bronchial versus C-fibres (e.g. histamine and serotonin have been reported to activate bronchial but not pulmonary C-fibres).

4. C-fibres and the local release of neuropeptides

The population of sensory nerves known as the chemosensitive C-fibres are thought to contain neuropeptides, in particular calcitonin gene-related peptide (CGRP) and the tachykinins substance P (SP) and neurokinin A (NKA). The stimulation of these nerve endings may result in a local or axon reflex and cause the release of the sensory neuropeptides contained within; this is the so-called 'efferent' function of sensory nerves [23] (Fig. 1). SP and NKA are potent inducers of airway smooth muscle contraction, vasodilatation, bronchial oedema and mucus hypersecretion, which are all classic symptoms of inflammatory airway diseases. Therefore, neuropeptides released from these nerve endings, via such a reflex, has been proposed to be important in the pathology of diseases such as asthma [23–25]. It has been demonstrated that electrically evoked bronchoconstriction in vitro and in vivo is blocked by NK_1 and NK_2 receptor antagonists [26–28]. Similarly, mucus secretion and microvascular leak seen in

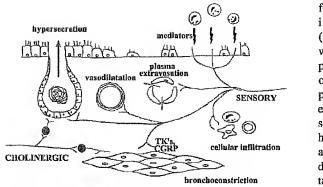

Fig. 1. In addition to their sensory function some primary afferent nerves may serve local efferent functions. Many C-fibres contain neuropeptides in their peripheral and central projections. In the CNS the neuropeptides act as neurotransmitters at the synapse between the primary afferent and secondary neurons. In the peripheral tissues the neuropeptides can be released locally as a consequence of axon reflexes leading to a series of events such as bronchoconstriction, increases in microvascular leakage and mucus secretion and termed 'neurogenic inflammation' (after Barnes et al., 1986 [23]).

the presence of muscarinic and adrenergic receptor blockade is inhibited by NK_2 receptor antagonists [26,29,30].

The relevance of neurogenic inflammation in human airways is not yet known. However, immunohistochemical studies have demonstrated the presence of SP-containing nerve fibres in human airways and the number and length of these fibres were found to be increased in the airways of asthmatics compared to normals [31]. However, other studies have failed to reproduce these findings. Although, functionally, non-adrenergic, non-cholinergic (NANC) contractile responses are weak in human airways in vitro [32], capsaicin-evoked mucus secretion has been demonstrated from isolated human airways [33]. These weak NANC responses and inconsistent peptide staining may be due to the degradation of peptides seen in denervated post-mortem tissue. Although it may be possible that such local reflexes can occur in the human lower airways, available data argue against this hypothesis. The ability of capsaicin to evoke contractions of human airway smooth muscle preparations and mucus secretion from human airway explants are unlikely to be mediated by neurokinins. Electrical field stimulation-induced contractions of and mucus secretion from human airways are likely to be completely abolished by atropine and neurokinin receptor antagonists have no effect on capsaicin-induced contractions of human airway preparations [34].

Recently, evidence was provided for NANC responses in humans in vivo with the demonstration that bradykinin-induced bronchoconstriction was blocked by a mixed NK_1/NK_2 receptor antagonist [35]. However, subsequent studies have shown that this antagonist did not prevent bronchoconstriction induced by NKA in asthmatics [36]. Potent, non-peptide, safe and selective NK_1 and NK_2 receptor antagonists have only recently become available for studies in humans. NKA-induced bronchospasm is inhibited by a selective NK_2 receptor antagonist (SR 48968C) [37]. However, when the same compound was administered orally for 9 days in allergic asthmatic patients, it did not have a significant bronchodilatory effect or a bronchoprotective effect against adenosine 5′-monophosphate hyperresponsiveness [38]. Further studies on the effects of these selective antagonists in asthma, and more specifically on other parameters such as allergen-induced hyperresponsiveness, and subjective asthma symptoms such as chest tightness and cough, should be studied before definitive conclusions can be drawn on the role of tachykinins in asthma. Furthermore, the contribution of different types of neurokinin receptor is unknown and it may be that studies with a combined NK_1 and NK_2 or even a NK_1, NK_2 and NK_3, receptor antagonist have to be carried out to demonstrate or rule out the possible role of these receptors in asthma.

5. Aδ versus C-fibres in evoking reflex events

Studies in vitro have shown that while Aδ-fibres innervating guinea-pig trachea may be stimulated by agents such as distilled water, hypertonic saline and, to a lesser extent, a low-chloride solution [17,39], they remain insensitive to chemical stimuli such as capsaicin, low pH solutions, bradykinin, histamine and 5-hydroxytryptamine [11]. Generally, higher concentrations of capsaicin are required to excite Aδ fibres [40]. This selectivity of capsaicin for C-fibres had led to the suggestion that these fibres may play a role in reflex events such as cough [41].

However, results obtained from in vivo experiments are at odds with these observations so that stimuli such as capsaicin, which in vitro are highly selective for C-fibres, do not observe this selectivity profile in vivo also stimulating Aδ fibres [42]. In particular, these studies have been criticised as examining only a limited population of Aδ-fibres located in the trachea and it may be that fibres in the lower airways are chemosensitive as has been described in vivo [43]. A large number of in vivo studies have indicated that RARs may be excited, whether directly or indirectly, by a variety of chemical stimuli including hyper- and hypo-osmolar solutions, low chloride, histamine, 5-HT, PGE_2, SP, cigarette smoke, ammonia and volatile anaesthetics. Nevertheless, whether direct or indirect, it may be accepted that RAR's do respond to chemical stimuli. This chemosensitivity coupled with their extreme mechanical sensitivity, responses to oedema and wide distribution at all airway levels implies that they play a major role in airway physiology and pathophysiology.

Experiments from Riccio et al. [44] in studies on guinea-pig airway afferent fibres may help to explain some of these apparent anomalies. It has been suggested in these studies that airway afferent fibres whose cell bodies reside in the nodose ganglia appear to correspond to myelinated fibres

involved in detecting normal physiological activity. These fibres are mechanoceptors that do not respond to chemical stimuli (e.g. capsaicin) [45]. In contrast, cell bodies of Aδ fibres and un-myelinated C-fibres are present in the jugular ganglia. Interestingly, these jugular afferent fibres are nociceptive in that they have reasonably high thresholds to mechanical stimuli but do respond to chemical stimuli such as capsaicin. However, this attempt to reconcile the discrepancy between results obtained in vivo in guinea-pigs and cats with those gathered in vitro using guinea-pig airway preparations seems unlikely since Riccio et al., noted that myelinated capsaicin-sensitive fibres are, like C-fibres in their preparation, relatively insensitive to mechanical stimulation and once activated, slowly adapting. This would make them unlikely to correspond to the RARs described in vivo. In fact, such afferents may be unique to the guinea-pig. With regard to other species, only 3/48 RARs responded (and just modestly) to capsaicin in rats [46] and capsaicin seems selective for C-fibres in dogs [47]. A more likely explanation might be that the secondary effects produced by C-fibre activation in cats and guinea-pigs in vivo (bronchospasm, mucus secretion, vascular engorgement, plasma exudation) leads to RAR activation. Thus, Bergren [48] showed that capsaicin- and bradykinin-evoked activation of RARs in guinea-pigs could be prevented by isoproterenol, indicating that the bronchospasm evoked by C-fibre activation evokes discharge in the mechanically sensitive RARs. Consistent with this notion of C-fibre mediated RAR activation, capsaicin-induced cough can be virtually abolished by inhaled neurokinin antagonists for the NK_1, NK_2 and NK_3 receptor. Such effects of inhaled neurokinin receptor antagonists [49,50], and systemically administered, low CNS penetrant compounds [51], in the guinea-pig cough model may argue for an indirect effect of capsaicin on RARs and an indirect role of C-fibres in cough. However, these reports are at odds with a study from Bolser et al. [52] who have demonstrated that neurokinin receptor antagonists inhibit cough induced by mechanical stimulation in cats via an effect on the central nervous system. On the basis of these results a central action of neurokinin receptor antagonists cannot be ruled out.

6. Role of Aδ and C-fibres in evoking central and local reflex events under inflammatory conditions

Sensory nerve activity may be enhanced during inflammation so that protective central and local reflexes become exacerbated and deleterious and may contribute to the pathophysiology and symptomatology of airway inflammatory diseases such as asthma and COPD [41,45]. Stimulation of sensory nerves can evoke bronchoconstriction, mucus secretion and cough via activation of an afferent central reflex pathway. Furthermore, activation of a particular sub set of sensory fibre, the C-fibres, is known to evoke neurogenic inflammation characterised by

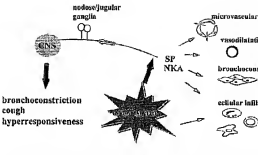

Fig. 2. Inflammatory mediators sensitise peripheral nociceptors which together with central sensitisation may lead to a variety of exaggerated functional responses such as bronchoconstriction, mucus secretion and cough via increases in both central and peripheral reflexes.

responses such as microvascular leakage of plasma proteins and cellular infiltrate into the airways [25].

The mechanism involved in the increased responsiveness of sensory nerves under inflammatory conditions has not been clearly defined but could involve the interaction of inflammatory mediators with sensory and autonomic nerves leading to an increase in the electrical activity of sensory afferent nerves and an increase in neurotransmitter release from autonomic nerves and C-fibre nerve endings. It has been suggested that this may be achieved by various mechanisms, which could be distinct, or act in combination such as the sensitisation of the peripheral sensory nerve endings or the central vagal neurones by these mediators, via the increased release of neuropeptides or by changes in the neuronal phenotype (Fig. 2).

6.1. Sensitisation of peripheral nerve endings

The sensitisation of nerve endings does not involve action potential discharge. However, the increase in membrane resistance and small depolarisation renders the nerve more excitable such that sub threshold stimuli may be sufficient for activation [53]. Using single fibre recording in guinea-pigs it has been demonstrated that airway sensory nerves may be acutely sensitised by inflammatory mediators such as PAF, PGI_2 and bradykinin. Thus, following perfusion of the receptive field of the innervated isolated trachea for 10 min, the responses of single C-fibres in response to either bradykinin (at concentrations that do not depolarise fibres per se) or capsaicin was significantly enhanced (degree of firing increased by 60–400%) [41,54,55]. Alternatively, in the same studies, the Aδ fibres did not exhibit this sensitisation phenomenon with no change in responses to hypertonic saline following pre-treatment of the fibre with BK, PGI_2 or PAF. However, other studies have demonstrated acute sensitisation of this fibre type after antigen challenge in trachea from sensitised guinea-pigs so that there was a reduction in the mechanical threshold [10].

As a functional correlate to the single fibre studies Fox et al. [55] examined the effects of several of these mediators on the cough reflex in conscious guinea-pigs. In these

studies animals were placed in a perspex box that allows free movement during exposure to aerosols. A cylinder supplied airflow into the box and changes in airflow induced by changes in respiration or coughing were detected by a pneumotachograph, amplified and recorded via a pressure transducer to a pen recorder. Cough sounds were amplified and recorded concurrently via a microphone placed in the box. Coughs were counted and recognised by the characteristic posture of the animal and the opening of the mouth, the sound produced and the airflow recordings distinguish cough from sneeze and augmented breaths. Exposure of guinea-pigs to aerosols of citric acid (low pH solution) or capsaicin produce reproducible cough presumably initiated by the activation of C-fibres (based on the single fibre data mentioned earlier [55], and possibly Aδ fibres. If these animals are exposed to bradykinin beforehand the cough response to citric acid was markedly enhanced. This enhancement was prevented by treatment with the B_2 receptor antagonist [55]. Bradykinin induced sensitisation of C-fibres maybe the mechanism operative in angiotensin converting enzyme (ACE) inhibitor-induced cough, where a proportion of patients receiving this treatment for hypertension or cardiac failure exhibit a chronic cough and increased cough sensitivity to citric acid or capsaicin. ACE (also known as kininase II) is an enzyme which is involved in the breakdown of bradykinin and therefore it has been hypothesised that the increased levels of bradykinin in the airways of patients on ACE inhibitor therapy could lead to the sensitisation of airway C-fibres leading to the enhanced sensitivity of the cough reflex. These findings suggest that airway sensory nerves, and in particular C-fibres, can be sensitised by inflammatory mediators. This phenomenon could contribute to the increased sensitivity of airway reflexes such as cough, mucus secretion and bronchoconstriction seen in airway inflammatory diseases.

6.2. Induction of tachykinin synthesis and enhanced release of tachykinins and the inflammatory response

Increased levels of neuropeptides are often found in airway tissues under pro-inflammatory conditions. For example the concentration of SP in the induced sputum from asthma and COPD patients was increased compared to that from normal volunteers [56]. However, data between different studies are variable with some studies describing elevated levels of tachykinins in inflammatory conditions, some showing no change [57] and some showing decreased levels of tachykinins [58]. However, these differences could be due to the variable degradation of these peptides. The situation is much clearer in 'the more controlled situation' of animal studies. Thus, 24 h after allergen challenge in sensitised guinea-pigs there was an increase in the amount of SP, NKA and CGRP immunoreactivity in the lung [59]. Under normal physiological conditions tachykinin-containing sensory nerves appear to be the C-fibres whose cell bodies reside in the jugular ganglia [45]. However, interestingly, following allergen challenge, nodose neurones whose axons projected to the airways were shown to express tachykinins. Functional studies support the idea that allergic inflammation causes phenotypic changes in the type of airway afferent fibres that contain neuropeptides such that non-nociceptive (capsaicin-insensitive mechanoceptors) as well as capsaicin-sensitive C-fibres contribute to the neuropeptide innervation [60]. The mechanisms involved in the phenotypic switching of these neurons is not known but neurotophic factors, whose expression is increased in airway inflammation [61–65], are thought to play a role.

7. Inhibition of sensory nerve function

Direct modulation of the activity of sensory nerves could conceivably inhibit airway effects resulting from both central and peripheral reflexes and may have advantages over other targets. Several studies have shown that NANC bronchoconstriction can be inhibited by activation of μ-opioid, $GABA_B$, α_2-adrenoceptors and NPY implying an inhibition of neuropeptide release from C-fibre nerve endings [66–69]. However, much less information is available on whether afferent activity of C- and or Aδ fibres may also be inhibited. Interestingly, it has been proposed that the inhibitory effect of these G-protein coupled receptors may be mediated through a common mechanism by opening of large conductance calcium-activated potassium (BK_{Ca}) channels [69] (Fig. 3). Activation of BK_{Ca} channels is thought to hyperpolarize sensory nerves reducing their excitability and a study, utilising a novel opener of this channel NS1619, has demonstrated an inhibition of NANC bronchoconstriction and firing of single C-fibres evoked by bradykinin in guinea-pig airways [70]. Furthermore,

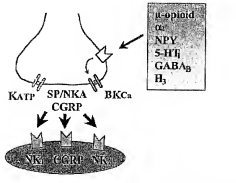

Fig. 3. Numerous studies have shown that NANC bronchoconstriction in guinea-pigs can be inhibited by activation of μ-opioid receptors, $GABA_B$ receptors, α_2-adrenoceptors, and neuropeptide Y receptors implying inhibition of neuropeptide release from sensory nerve endings. Much less information is available on whether the afferent activity of sensory nerves may also be inhibited. It has been proposed that the inhibitory effect of these receptors may be mediated through a common mechanism by opening of large conductance calcium activated potassium channel (BK_{Ca}) [69,70].

NS1619 also inhibited Aδ fibre firing evoked by distilled water and the selective BK_{Ca} channel blocker, iberiotoxin, inhibited all the actions of NS1619. Importantly, this compound also inhibited sensory nerve reflexes in vivo in this case citric acid induced cough in the guinea-pig [70].

8. Conclusion

In conclusion, there appears to be a certain amount of redundancy with regard to the role of different populations of sensory fibre innervating the airways. For example, recent evidence suggests that both C-and Aδ fibres are mechanically and chemosensitive (via either a direct or indirect effect of inflammatory mediators) and both can be sensitised leading to exaggerated functional responses such as cough. Furthermore, several agents that can inhibit sensory nerve activity peripherally and thereby suppress the cough reflex (e.g. local anaesthetics, NS1619) may have a more generalised action via inhibition of both myelinated and unmyelinated fibres. Furthermore, with regard to neurogenic inflammation and the release or neuropeptides from sensory afferents it would appear that C-fibres, and possibly Aδ-fibres following allergic inflammation, play a major role. In summary, the weight of evidence suggests an important role for both fibre types in both the defensive reflexes and in the prolonged sensitisation of fibres that may occur in inflammatory conditions leading to the generation of inappropriate and harmful reflex actions.

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Exhibit AB

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Title:	**Capsazepine as a selective antagonist of capsaicin-induced activation of C-fibres in guinea-pig bronchi.**
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Short communication

Capsazepine as a selective antagonist of capsaicin-induced activation of C-fibres in guinea-pig bronchi

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We investigated the action of capsazepine, an antagonist of the actions of capsaicin on sensory neurones, on the contractile responses evoked by capsaicin or by electrical field stimulation (EFS) in guinea-pig bronchi. Capsazepine (10^{-5} M) selectively inhibited responses to capsaicin, producing a significant change in EC_{50} values but not the Hill coefficient (n_H), suggesting that capsazepine acts as a competitive antagonist (apparent $pK_B = 5.12$) whereas ruthenium red is a non-competitive antagonist. Capsazepine and ruthenium red were without effect on EFS-induced responses.

Capsazepine; Non-adrenergic non-cholinergic (NANC) excitatory nerves; Capsaicin; Electrical field stimulation; Tetrodotoxin

1. Introduction

Capsaicin (N-methyl-N-vanillyl-6-nonenamide), the pungent ingredient of red peppers, selectively activates a population of primary afferent sensory neurones by interacting with a specific receptor to open a membrane ion channel that is permeable to both monovalent and divalent cations (Bevan and Szolcsanyi, 1990). In guinea-pig bronchi these nerves can be stimulated by electrical field stimulation (EFS) or with capsaicin to produce a contractile response mediated via the release of sensory neuropeptides such as substance P and neurokinin A (Lundberg and Saria, 1982, 1987).

Ruthenium red has been used in previous studies as a capsaicin antagonist which has been demonstrated to block the contractile actions of capsaicin on guinea-pig bronchi with no effect on neurally evoked excitatory non-adrenergic non-cholinergic (e-NANC) contractile responses (Maggi et al., 1989). However, this compound is not very specific and may have effects on other membrane receptors and ion channels (Bevan et al., 1991; Maggi et al., 1989).

In this study we have characterised the action of a new compound, capsazepine (2-[2-(4-chlorophenyl) ethyl-aminothiocarbonyl]-7,8-dihydroxy-2,3,4,5,-tetrahydro-1H-2-benzazepine), that acts as a selective (Dickenson and Dray, 1991; Dray et al., 1991) and competitive antagonist of the actions of capsaicin on sensory neurones (Bevan et al., 1991), in guinea-pig bronchi.

2. Materials and methods

Male Dunkin-Hartley guinea pigs (250–500 g) were killed by cervical dislocation. The lungs with the bronchi and trachea were removed and placed in Krebs–Henseleit (KH) solution of the following composition (mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 25.5 and glucose 5.6; it was gassed continuously with 95% O_2 and 5% CO_2 to give pH 7.4. The parenchyma was dissected away to reveal two main and two hilar bronchi, which were suspended between platinum wire field electrodes in 10 ml organ baths containing KH solution. Indomethacin (10^{-5} M) was present throughout in order to prevent fading of neural responses due to endogenous prostaglandin production. The tissues were allowed to equilibrate for 1 h, with washing, under a resting tension of 500 mg for main and hilar bronchi, which was found to be optimal for measuring changes in tension. Isometric contractile responses were measured using Grass FT. 03 force-displacement transducers and recorded on a polygraph.

In the presence of atropine and propranolol (both 10^{-6} M), the effect of capsazepine and ruthenium red (10^{-5} M) on the NANC constrictor responses elicited to electrical field stimulation (EFS) were studied in main and hilar bronchi. Biphasic square-wave pulses were delivered for 20 s periods from a Grass S88

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stimulator using a supramaximal voltage of 40 V at source and a pulse duration of 0–5 ms. A frequency of 8 Hz was used, which was found to be the frequency which produces an approximate 50% maximal response to EFS in these tissues. The tissues were stimulated every 30 min or when the response had returned to its basal level. At least three consistent EFS responses were obtained to check the reproducibility of the response, and tissues giving variable results were not studied. Drugs were added 20 min before stimulation.

In separate experiments the effect of capsazepine (10^{-7}–10^{-4} M) and ruthenium red (10^{-5} M) on cumulative concentration–response relationships to capsaicin (10^{-8}–3×10^{-5} M) was studied. The results were expressed as a percentage of the maximal contractile response to histamine (10^{-3} M) determined in each ring.

All data in the text are means ± S.E.M. of isometric contractile responses measured and expressed in g. Statistical analysis was performed by means of Student's t-test for paired data (contraction due to EFS) or by Student's t-test for unpaired data (contraction due to capsaicin). EC_{50} values were calculated by using a non-linear iterative curve fitting program, Inplot (Graphpad Inc. CA, U.S.A.).

All drugs used were obtained from Sigma except (2-[2-(4-chlorophenyl)ethylamino-thiocarbonyl]-7,8-dihydroxy-2,3,4,5-tetrahydro-1H-2-benzazepine) (capsazepine) which was kindly donated by the Sandoz Institute.

3. Results

Neither capsazepine nor ruthenium red (both 10^{-5} M) had any effect on resting tone in guinea-pig bronchi or had any significant effect on e-NANC neurally evoked responses (0.67 ± 0.12 g in the absence and 0.66 ± 0.14 g in the presence of capsazepine; 0.47 ± 0.09 g in the absence and 0.47 ± 0.07 g in the presence of ruthenium red, n = 5) (fig. 1B).

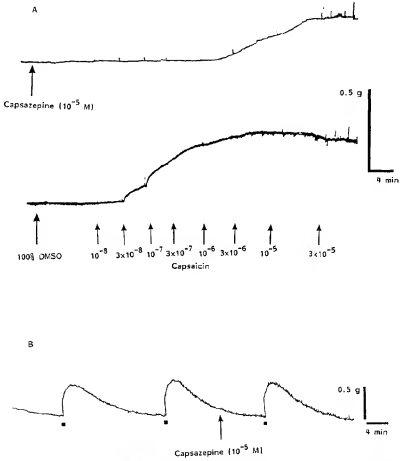

Fig. 1. (A) Typical traces of cumulative contractile responses to capsaicin (10^{-8}–3×10^{-5} M) in the presence of capsazepine (10^{-5} M) or vehicle (100% DMSO) in guinea-pig main and hilar bronchi. (B) Typical traces of excitatory non-adrenergic non-cholinergic (e-NANC) contractile responses to EFS (■) (40 V, 0.5 ms, 8 Hz for 20 s) in guinea-pig main and hilar bronchi before and after the addition of capsazepine (10^{-5} M).

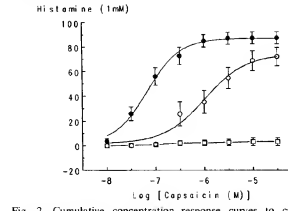

Fig. 2. Cumulative concentration–response curves to capsaicin (10^{-8}–3×10^{-5} M) (●) in the presence of capsazepine (10^{-5} M) (○) and ruthenium red (10^{-5} M) (□) expressed as a % of the maximum contraction to histamine (1 mM). Each point represents mean ± S.E.M. of 5–13 observations.

Low concentrations capsazepine (10^{-7} and 10^{-6} M) had no significant effect on cumulative responses to capsaicin (10^{-8}–3×10^{-5} M). A higher concentration of capsazepine (10^{-5} M) caused a parallel right ward shift ($n_H = 1.5 \pm 0.34$ (n = 5) in the presence of capsazepine and $n_H = 1.85 \pm 0.2$ in the absence (n = 13), NS) in capsaicin concentration–response curves ($EC_{50} = 1.3 \times 10^{-6} \pm 4.1$ M in the presence and $EC_{50} = 1.1 \times 10^{-7} \pm 2.9$ M in the absence of capsazepine, $P < 0.01$) (fig. 2). However, there was no significant reduction in the maximum response to capsaicin (0.99 ± 0.09 g in the absence and 0.8 ± 0.2 g in the presence of capsazepine) (fig. 2). These results suggest that capsazepine (10^{-5} M) acts as a competitive antagonist. Application of the Schild equation gave a K_B value of 0.75 μM corresponding to pK_B of 5.12. The maximum contractile response to histamine (10^{-3} M) was not significantly altered (1.2 ± 0.18 g in the absence and 1.1 ± 0.16 g in the presence of capsazepine). At a concentration of capsaicin (10^{-7} M) that produced an equivalent size contraction as the neurally evoked e-NANC response (0.7 ± 0.12 g compared with 0.64 ± 0.11 g, respectively) capsazepine (10^{-5} M) produced 98.4% inhibition ($P < 0.001$) of the response to capsaicin with no effect on the neurally evoked response (fig. 1A and B).

Ruthenium red (10^{-5} M) completely inhibited contractile responses to capsaicin (10^{-8}–3×10^{-5} M).

4. Discussion

Previous studies have demonstrated that there are at least two mechanisms that can induce release of neuropeptides from capsaicin-sensitive primary sensory neurones. Neurotransmitters can be released by depolarising stimuli such as EFS which activates voltage-sensitive calcium channels (ω-conotoxin sensitive, N-type voltage-sensitive channels). Secondly, they may be released by capsaicin in a TTX-insensitive manner and unaffected by ω-conotoxin. Capsaicin is reported to act on a putative membrane receptor to open an ion channel that is permeable to both monovalent and divalent cations (Bevan and Szolcsanyi, 1990).

Recent studies have shown that capsaicin-induced contractile effects in guinea-pig bronchi may be abolished by ruthenium red without any effect on neurally evoked (e-NANC) contractile responses to EFS (Maggi et al., 1989). Ruthenium red also selectively inhibits capsaicin- and citric acid-induced excitation of sensory nerves in guinea-pig lung as demonstrated by calcitonin gene-related peptide-like immunoreactivity release, bronchoconstriction and coughing without affecting bronchoconstriction induced by vagal nerve stimulation (Amann et al., 1989; Lou et al., 1991). However, ruthenium red is not a specific antagonist and can affect other membrane receptors and ion channels (Bevan et al., 1991).

In this study we describe the action of a newly developed capsaicin antagonist, capsazepine. This compound has been demonstrated to act as a potent and specific competitive antagonist of capsaicin-induced activation of sensory C-fibres in a number of preparations (Bevan et al., 1991; Dray et al., 1991; Dickenson and Dray, 1991).

The present study suggests that capsazepine has no effect on electrically evoked e-NANC contractions of guinea-pig bronchi in a similar manner as ruthenium red (Maggi et al., 1989). Capsazepine (10^{-5} M) caused a rightward parallel shift in the capsaicin concentration–response curve. There was no significant difference between the Hill coefficients of the concentration–response curves obtained in the presence and absence of capsazepine but, the coefficients were not close to 1 presumably because the concentration–response curves were artificially steep due to depletion of neuropeptides before addition of high doses of capsaicin. There was a significant ($P < 0.01$) shift in the response curves to capsaicin ($EC_{50} = 1.3 \times 10^{-6} \pm 4.1$ M in the presence and $EC_{50} = 1.1 \times 10^{-7} \pm 2.9$ M in the absence of capsazepine). In addition, capsazepine did not reduce the maximum response obtained to capsaicin. These results suggest that capsazepine (10^{-5} M) is acting as a competitive antagonist (with an apparent pK_B of 5.12). We were not able to perform Schild analysis of this data and thereby obtain a pA_2 value as we did not have data on capsaicin in the presence of three effective doses of antagonist. The action of capsazepine appeared to be selective as maximum contractile responses to histamine were not affected. In comparison ruthenium red (10^{-5} M) completely inhib-

ited contractions to capsaicin suggestive of non-competitive antagonism. However, neither capsazepine nor ruthenium red inhibited EFS-induced contractile responses. Although for a concentration of capsaicin which mimicked the contractile response to EFS there was virtually complete inhibition.

This suggests that ruthenium red and capsazepine act by affecting the opening of an ion channel by capsaicin without affecting responses evoked by EFS which activates voltage sensitive calcium channels. However, where capsazepine seems to be acting as a competitive antagonist ruthenium red acts in a non-competitive fashion. This suggests that capsazepine may be acting as a capsaicin receptor antagonist whereas ruthenium red is not very specific and may have effects on other membrane receptors and ion channels (Maggi et al., 1989).

In conclusion, this data, supports the fact that EFS and capsaicin both release neuropeptides from sensory nerves by different mechanisms. Capsazepine is the first competitive antagonist to be developed and maybe a better compound to study the receptor-mediated effects of capsaicin than ruthenium red.

Acknowledgements

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Exhibit AC

Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea pigs

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Lalloo, Umesh G., Alyson J. Fox, Maria G. Belvisi, K. Fan Chung, and Peter J. Barnes. Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea pigs. *J. Appl. Physiol.* 79(4): 1082–1087, 1995.—Acidic solutions mimick many of the effects of capsaicin (Cap), including pain, bronchoconstriction, cough, and sensory neuropeptide release. Evidence from the use of the Cap antagonist capsazepine suggests that in some cases protons act at the Cap receptor. In the present study, we have investigated whether cough evoked by Cap and citric acid (CA) is mediated specifically via the Cap receptor on airway sensory nerves. We have examined the effects of capsazepine on Cap-, CA-, and hypertonic saline-induced cough and also on CA-induced nasal irritation in awake guinea pigs. Capsazepine was nebulized for 5 min before cough challenges with Cap for 5 min and CA for 10 min. Control animals were pretreated with vehicle alone. Capsazepine (100 μM) inhibited the cough response to 30 μM Cap from 0.77 ± 0.14 coughs/min in control animals to 0.23 ± 0.08 coughs/min ($P < 0.05$) and to 80 μM Cap from 1.4 ± 0.23 to 0.3 ± 0.11 coughs/min ($P < 0.01$). There was no effect, however, of lower concentrations of capsazepine (5 and 10 μM) against Cap-evoked cough. At a concentration of 100 μM, capsazepine also inhibited the coughing induced by 0.25 M CA from 1.8 ± 0.26 to 0.93 ± 0.31 coughs/min ($P < 0.05$) but not that induced by 0.5 M CA. Nasal irritation induced by 0.25 M CA, but not by 0.5 M CA, was also inhibited by capsazepine from 2.47 ± 0.37 to 0.75 ± 0.31 nose wipes/min ($P < 0.05$). This inhibitory effect of capsazepine did not appear to reflect a nonspecific suppression of the cough reflex, since cough evoked by exposure to hypertonic (7%) saline for 10 min was unaffected by pretreatment with capsazepine (100 μM). These data show that capsazepine is a specific inhibitor of Cap- and CA-induced cough in guinea pigs. Moreover, they suggest that low pH stimuli evoke cough and nasal irritation by an action at the Cap receptor, either directly or through the release of an intermediate agent.

capsaicin receptor; low pH; cough; airway sensory nerves

COUGH IS A POWERFUL DEFENSIVE REFLEX that may be elicited by the activation of myelinated and nonmyelinated afferent fibers distributed throughout the larynx and tracheobronchial tree (21). Capsaicin is a potent tussive agent in humans and guinea pigs (9, 13, 14, 21) and it also evokes other effects, such as bronchoconstriction (14, 19), plasma extravasation (25), and mucus secretion in the airways (22). These effects are mediated by selective excitation of a subpopulation of primary afferent neurons (7), and in the guinea pig airways, capsaicin has been shown to excite nonmyelinated C fibers but not myelinated Aδ-fibers (16). At a cellular level, this excitation results from the opening of a relatively nonselective cation channel and subsequent activation of an inward depolarizing current (3). The inorganic dye ruthenium red blocks this channel (11), and this may underlie its ability to act as a noncompetitive antagonist at certain concentrations (1). The recent development of the capsaicin antagonist capsazepine has provided firm evidence that capsaicin interacts with a specific membrane-bound receptor (5), the molecular identity of which remains unclear. Capsazepine also inhibits the binding of the potent capsaicin analogue resiniferatoxin in the spinal cord and peripheral tissues, including the airways (29), and it has been used to further investigate the mechanism of action of capsaicin in the airways. Thus it competitively antagonizes the contractile effects of capsaicin in the airways of isolated guinea pig bronchi (2, 12) and inhibits capsaicin-evoked bronchoconstriction in guinea pigs in vivo (27).

Many of the airway effects of capsaicin are mimicked by citric acid. Thus citric acid is also a potent tussive agent in humans and guinea pigs (14, 28) and it evokes bronchoconstriction and sensory neuropeptide release from perfused lung (24, 27). Interestingly, these effects of citric acid in the guinea pig are inhibited both by capsaicin pretreatment (13, 27) and by ruthenium red (23), suggesting a similar mechanism of action to capsaicin. This is supported by the findings that protons and capsaicin excite the same sensory fibers in guinea pig airways (17) and, in addition, open the same population of ion channels in rat isolated dorsal root ganglion neurons (4). There is evidence, therefore, that protons and capsaicin act at the same site on sensory neurons, and this is supported by studies showing that capsazepine inhibits proton-evoked excitation of airway C fibers (17) and citric acid-evoked bronchoconstriction in guinea pigs (24, 27). In contrast, however, it is without effect against proton-evoked excitation of other sensory neurons, such as rat isolated dorsal root ganglion neurons and vagus nerve (5, 6).

In the present study, we have tested the hypothesis that both capsaicin and protons stimulate the cough reflex through an action at the capsaicin receptor. We have compared the effects of capsazepine against cough and nasal irritation in the guinea pig, evoked by citric acid, capsaicin, and hypertonic saline.

METHODS

Animals. Male Dunkin-Hartley guinea pigs (300–450 g) were acclimatized in the animal facility for 1 wk after delivery from the breeders (Harlan UK, Oxon, UK) before the study.

Cough challenges. The guinea pigs were placed in a transparent perspex box 20 × 10 × 10 cm, which allowed free movement of the guinea pigs for the cough challenges and

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TABLE 1. *Effect of terbutaline pretreatment on time to onset of respiratory distress and cough with different tussive stimuli*

	Time to Respiratory Distress, s			Cough Response, coughs/min		
	Citric acid	Capsaicin	Hypertonic saline	Citric acid	Capsaicin	Hypertonic saline
Saline	107±28	156±74.5	ND	3.3±0.18	1.6±0.4	0.5±0.1
Terbutaline	495±88 ($P < 0.05$)	275±39 ($P < 0.01$)	ND	2.7±0.23 NS	1.3±0.3 NS	0.6±0.2 NS

Table shows effect of pretreatment of animals with 0.05 mg/kg terbutaline on time to onset of respiratory distress and cough after challenge with citric acid (0.5 M), capsaicin (30 μM), and hypertonic saline. Hypertonic saline did not induce respiratory distress, hence this parameter was not determined (ND). In each case, data are presented from groups of 6 or 7 animals. NS, not significant at the 95% level. P values refer to significance of difference between saline- and terbutaline-treated groups.

exposure to capsazepine aerosols. Airflow into the box was provided at 600 ml/min through one end and out the opposite end. Changes in airflow induced by the animals' respiration and coughing were recorded by a pneumotachograph (Fleisch 000, Switzerland) connected to the box above the air entry port. Flow changes were amplified and recorded by a pressure transducer (Furness Controls, Bexhill, UK) and recorded onto a moving pen recorder (Lectromed, St. Peter, Jersey, Channel Islands, UK). The cough sounds were amplified by a lapel microphone (model ECM T7, Sony, Japan) placed in the box and recorded by a transducer onto moving pen recorder. All solutions were nebulized via a mini-ultrasonic nebulizer (Pulmisonic, DeVilbiss, Somerset, PA; aerodynamic mean mass median particle diameter 0.9 μm, with an output of 0.4 ml/min) connected to the airflow port. Coughs were counted by a trained observer and recognized from the characteristic posture of the animal, the sound produced, and the flow and sound transducer recordings. Coughs were readily distinguished from sneezes.

Study protocols. All studies were carried out at the same time of day. For each experiment, a control group of animals from the same cage was used on a rolling protocol to control for any changes in the sensitivity of the cough reflex and experimental conditions. Animals were allowed at least 7 days rest between experiments to allow complete recovery, and each group of animals was used only twice, being exposed to the same stimulus. This avoided tachyphylaxis, since in preliminary experiments we found that cough responses to 0.5 M citric acid were reproducible when repeated in the same group of animals 3 days after the first challenge (*day 1* = 1.82 ± 0.25 coughs/min, *day 3* = 1.77 ± 0.31 coughs/min, $P > 0.05$). In most experiments, each group contained six animals.

Before the cough challenges with citric acid and capsaicin, all animals were pretreated with 0.05 mg/kg terbutaline sulfate (0.18 μmol/kg) administered intraperitoneally 3 min before cough challenges to minimize respiratory distress. This pretreatment did not affect cough responses to any of the challenges used in the present study but it significantly prolonged the time to respiratory distress, thereby allowing coughs to be counted for up to 10 min with the different challenges (see Table 1 and RESULTS). After pretreatment with terbutaline sulfate, the animals were placed in the perspex box and allowed 3 min to acclimatize. They were then exposed to capsazepine or vehicle for 5 min, followed by a citric acid or capsaicin cough challenge in the continued presence of capsazepine or vehicle. Citric acid cough challenges were performed by using 0.25 or 0.5 M citric acid nebulized for 10 min, whereas capsaicin cough challenges were performed by using a 30- or 80-μM solution, nebulized for 5 min. The coughs were counted during the period of nebulization for both stimuli. During the 10-min citric acid challenges, the number of times the animals wiped their nose with their forepaws was also counted and used as an indirect index of nasal irritation (24).

To obtain a suitable cough challenge to examine any nonspecific inhibitory effect of 100 μM capsazepine on the cough reflex, we also investigated whether an isotonic chloride-deficient solution of 1.26% sodium bicarbonate (15 mM, pH 8) and hypertonic saline (7% sodium chloride, 1.2 M) would evoke cough. We have recently shown that chloride-deficient solutions and hypertonic saline excite both Aδ- and C fibers innervating the guinea-pig airways (15), and it was anticipated, therefore, that these stimuli would also evoke cough. The chloride-deficient solution and hypertonic saline were both nebulized for 10 min. Hypertonic saline was found to induce cough in our model (see RESULTS), whereas the chloride-deficient solution failed to do so. Hypertonic saline did not induce respiratory distress, but to maintian consistency in the protocols we pretreated the guinea pigs with 0.05 mg/kg ip terbutaline 3 min before the cough challenges.

Drugs. Capsazepine [2-[2-(4-chlorophenyl)ethyl aminothiocarbonyl]-7,8-dihydroxy-2,3,4,5-tetrahydro-1H-2-enzazepine], kindly supplied by Dr. Stuart Bevan, Sandoz Institute for Medical Research, London, UK, was prepared from a stock solution of 100 mM in dimethyl sulfoxide and diluted to 10 mM in a 0.9% NaCl solution (150 mM) containing 10% Tween 80 and 10% alcohol and further diluted in 0.9% NaCl to 5, 10, and 100 μM. The vehicle for capsazepine was the control solution. Citric acid (Sigma Chemical, Poole, UK) was made up to 0.25 and 0.5 M in the capsazepine solution or vehicle. Capsaicin (Sigma Chemical) was made up to 30 and 80 μM in the capsazepine solution or vehicle from a 100-mM stock solution in ethanol. Citric acid and capsaicin for the cough challenges were dissolved in the solution containing capsazepine or vehicle that was used to pretreat the guinea pig.

Statistical analysis. The number of coughs and nose wipes have been presented as the mean number of coughs or nose wipes per minute ± SE of the mean. The nonparametric Mann-Whitney two-sample test was used to evaluate the effect of capsazepine, since a normal distribution of coughs was not assumed given the small sample sizes ($n = 4-7$). $P < 0.05$ was considered significant.

RESULTS

Capsaicin-induced cough. The cough responses induced by 30 and 80 μM capsaicin were consistent within groups of guinea pigs, although there was some variation between groups. These concentrations were used as standard in the present study, since at concentrations <30 μM the cough responses were small and extremely variable, whereas concentrations >80 μM caused severe respiratory distress despite terbutaline pretreatment. After pretreatment with terbutaline, the

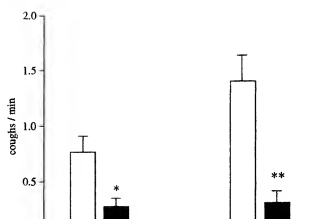

FIG. 1. Effect of capsazepine on cough induced by nebulization with capsaicin for 5 min. Guinea pigs were pretreated with vehicle (open bars) or capsazepine 100 μM (filled bars) for 5 min before capsaicin challenge. Each column represents mean ± SE for 6 animals. * $P < 0.05$, ** $P < 0.01$.

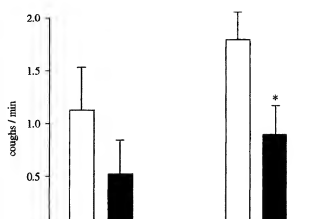

FIG. 2. Effect of capsazepine on cough induced by 0.25 M citric acid nebulized for 10 min. Guinea pigs were pretreated with vehicle (open bars) or capsazepine (filled bars) for 5 min before challenge with citric acid. Each column represents mean ± SE for 6 animals. * $P < 0.05$.

time to onset of respiratory distress after exposure to 30 μM capsaicin was significantly increased, whereas the cough response was unaffected (Table 1).

In the first experiment, exposure of control animals to the vehicle solution for 5 min produced 1.6 ± 0.64 coughs/min after challenge with 30 μM capsaicin. There was no inhibition of this response with 5 μM capsazepine (1.5 ± 0.72 coughs/min; $n = 4$) or 10 μM capsazepine (2.2 ± 0.48 coughs/min; $n = 4$). Additionally, exposure to the capsazepine aerosol for 10 min made no difference. However, coughing induced by 30 μM capsaicin was significantly inhibited from 0.77 ± 0.14 to 0.27 ± 0.08 coughs/min ($n = 6$ each) in the presence of 100 μM capsazepine ($P < 0.05$) (Fig. 1). The cough response to 80 μM capsaicin (1.4 ± 0.23 coughs/min) was similarly inhibited in the presence of 100 μM capsazepine (0.3 ± 0.11 coughs/min; $n = 6$ each; $P < 0.01$) (Fig. 1).

Citric acid-induced cough and nasal irritation. Exposure to citric acid aerosol for 10 min tended to induce a greater number of coughs than that to capsaicin. As with capsaicin challenge, terbutaline pretreatment increased the time to respiratory distress after 0.5 M citric acid challenge, but the cough response was unaffected (Table 1). For the capsazepine study, exposure to 0.5 M citric acid elicited a cough response of 1.87 ± 0.49 coughs/min in control animals ($n = 6$) after exposure to the vehicle solution. This response was not inhibited by 5 or 10 μM capsazepine (1.4 ± 0.6 and 1.32 ± 0.25 coughs/min, respectively; $n = 6$ each). In a separate series of experiments, the control response to 0.5 M citric acid (1.33 ± 0.33 coughs/min) was again not different to that seen in animals pretreated with 100 μM capsazepine (1.6 ± 0.34 coughs/min; $n = 6$). However, with a lower concentration of citric acid (0.25 M) there was a significant inhibition in coughs after exposure to 100 μM capsazepine, from 1.8 ± 0.26 to 0.93 ± 0.31 coughs/min ($n = 6$ each; $P < 0.05$) (Fig. 2). A lower concentration of capsazepine (10 μM) showed some inhibition of the cough response to 0.25 M citric acid, but this was not significant ($P = 0.14$; Fig. 2). Capsazepine (100 μM) also significantly inhibited the nasal irritation induced by 0.25 M of citric acid, from 2.47 ± 0.37 to 0.75 ± 0.31 nose wipes/min ($P < 0.05$; Fig 3). Again, 10 μM of capsazepine reduced the nasal irritation, but this was not significant ($P = 0.58$; Fig. 3). The nasal irritation produced by 0.5 M citric acid challenge was not inhibited by 100 μM capsazepine (control 5.0 ± 1.09 nose wipes/min; capsazepine 4.3 ± 1.27 nose wipes/min).

The tussive effect of citric acid was due to the reduction in pH rather than to the citrate anion, since in a separate series of experiments exposure of guinea pigs to 0.5 M sodium citrate (pH 8.53) or physiological saline (pH 5.17) aerosols for 10 min ($n = 6$ each) produced no significant cough response, whereas the response to 0.5 M citric acid (pH 1.07) was 1.77 ± 0.31 coughs /min.

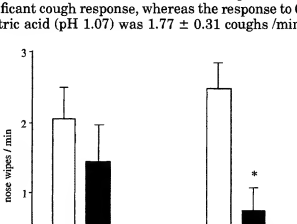

FIG. 3. Effect of capsazepine on nasal irritation induced by 0.25 M citric acid nebulization for 10 min. Nasal irritation was assessed as no. of times animals wiped their noses with their forepaws during 10-min citric acid cough challenge. Guinea pigs were pretreated with vehicle (open bars) or capsazepine (filled bars) for 5 min before challenge with citric acid. Each column represents mean ± SE for 6 animals. * $P < 0.05$.

Hypertonic sline-induced coughs. Exposure of the animals to the chloride-deficient solution aerosol (1.26% sodium bicarbonate) failed to induce a significant cough response and was not different from saline. However, exposure of the animals to hypertonic (7%) saline for 10 min produced a significant cough response (0.68 ± 0.11 coughs/min; $n = 4$). Interestingly, the guinea pigs did not develop significant respiratory distress, but terbutaline pretreatment was used to maintain consistency in the protocols and was found not to affect the cough response to hypertonic saline (Table 1). We therefore used the hypertonic saline-induced cough challenge to test whether 100 μM capsazepine had any nonspecific inhibitory effect on the cough reflex. The cough response in animals pretreated with vehicle (0.48 ± 0.07 coughs/min) was not significantly different from that seen in animals pretreated with 100 μM capsazepine (0.52 ± 0.12 coughs/min, $n = 6$ each; $P > 0.05$).

DISCUSSION

In the present study, we have shown that cough evoked by capsaicin and citric acid, but not that evoked by hypertonic saline, is mediated via an interaction with the capsaicin receptor on airway sensory nerves. It has been previously shown that the cough and bronchoconstrictor response to citric acid, like that of capsaicin, is abolished by systemic pretreatment with capsaicin (13). This implies that both agents act on the same population of primary afferent neurons and, indeed, single-fiber recording studies have shown that capsaicin and low pH solutions selectively excite tracheal C fibers but not Aδ-fibers in the guinea pig (17).

The sensory neuron excitatory effects of capsaicin have been proposed to be mediated via a specific capsazepine-sensitive receptor site, and, as expected, capsazepine inhibited cough evoked by capsaicin. In addition, however, citric acid-induced cough and nasal irritation were also inhibited. This extends previous findings on the activity of protons in the airway, since it has been previously shown that capsazepine inhibits low pH-evoked bronchoconstriction (24, 27) as well as firing of single C fibers (17). This suggests a common mechanism of action of protons and capsaicin at the capsaicin receptor on afferent fibers and is supported by a similar inhibition of low pH activation of sensory nerves in other tissues, such as rat soleus (26), guinea pig nose (24), and guinea pig heart (18). This is in contrast to the findings in other preparations, such as rat vagus and rat isolated dorsal root ganglion neurons, where protons and capsaicin have been shown to act at the same channel, that proton-induced activation is not blocked by capsazepine at concentrations that effectively block capsaicin (5, 6). The inhibitory effect seen in guinea pig airway and other tissues may reflect the release of some other factor by protons, from one of the many cell types present, which then acts on the capsazepine-sensitive site (see Refs. 17, 26, and 27). Alternatively, the varying effects in different tissues may reflect the existence of subtypes of the capsaicin receptor. It is worth remembering, however, that cough is a reflex response and as such has several components between initial excitation of the airway sensory receptor and final effector mechanism. In addition, although single-fiber experiments have characterized the sensory receptor populations in the guinea pig trachea and main bronchi (15, 16), the properties of those lower down the airway are unknown, as well as how these different fiber types might interact to affect reflex responses. These considerations should be borne in mind when comparing data obtained from in vitro and in vivo studies.

We found that capsazepine inhibited cough evoked by 0.25 but not 0.5 M citric acid. This may seem surprising, since the cough response to both concentrations was rather similar, although a direct comparison is difficult, because the data for each concentration were obtained from different groups of animals. It is possible that 0.5 M citric acid is near the top of the dose-response curve, such that any antagonism by capsazepine would not be apparent. However, it may be noted that ruthenium red has also been reported to inhibit cough evoked by low but not higher concentrations of citric acid (8). Ruthenium red appears to inhibit capsaicin and low pH excitation of sensory nerves by blocking the calcium channel associated with the capsaicin receptor (1). It is possible that at higher concentrations of citric acid, sensory nerves are excited by a nonspecific action of protons on membrane channel activity, whereas at lower concentrations there is the release of some other mediator, which acts, like capsaicin, at the capsaicin receptor and which is blocked by capsazepine and ruthenium red.

We also confirmed the observation of Lou and Lundberg (24) that capsazepine inhibited the nasal irritation induced by citric acid. In the present study, only the nasal irritation induced by 0.25 M citric acid was inhibited by 100 μM capsazepine. Lou and Lundberg induced nasal irritation by direct instillation of 25 μl of 0.4 M citric acid and 5 μM capsaicin into the guinea pigs nostrils. The addition of 100 μM capsazepine to the solutions inhibited the nasal irritation to these two irritants but not to nicotine. This confirms the common mechanism of action of protons and capsaicin and indicates that capsazepine at a concentration of 100 μM still functions as a specific capsaicin-receptor antagonist.

The concentration of capsazepine required to inhibit capsaicin and citric acid cough in the present study was relatively high, and previous studies examining the inhibitory effects of capsazepine on capsaicin-induced airway smooth muscle contraction have shown a significant response at concentrations of 5 and 10 μM (12, 27). However, administration of substances to the airway by nebulization in awake guinea pigs is likely to require higher concentrations than those required by administration via endotracheal tube or direct application to tissues in vitro. This may explain the higher concentration of capsazepine that is required to inhibit the cough response. For example, capsaicin stimulates firing of all C fibers in an in vitro preparation of the vagus nerve with the trachea and main bronchi of the guinea pig at concentrations ranging from 30 nM to 3 μM (16), whereas concentrations of 30 μM or greater are required to produce tussive responses when admin-

istered by nebulization in awake guinea pigs, an observation confirmed by others (8, 13, 14). Such high concentrations of agonist would require correspondingly high concentrations of antagonist.

A high concentration of capsazepine may also have nonspecific inhibitory effects, and, indeed, it has been reported to block neuronal calcium channels at high concentrations (10). This action may therefore underlie the reported inhibition by capsazepine of proton-evoked calcium-dependent neuropeptide release (18, 24) and, in particular, inhibition of potassium chloride-evoked neuropeptide release from rat soleus muscle (26). In this regard, it is perhaps significant that calcium channel antagonists inhibit cough, an effect considered to be mediated via inhibition of *N*-methyl-D-aspartic acid-activated calcium channels in the "cough center" (20a). It is possible therefore that capsazepine, if it reached sufficient systemic concentrations after inhalation, could inhibit the cough reflex by a block of central calcium channels. However, such nonspecific effects are unlikely to account for the inhibitory actions of capsazepine seen in the present study, since blockade of calcium channels would have little effect on afferent impulses generated in the airway sensory nerves. More importantly, our finding that hypertonic saline-induced cough was not inhibited by 100 μM capsazepine precludes a nonspecific inhibitory action, either on airway sensory nerves or on central pathways. Single-fiber recording studies have shown that hypertonic (7%) saline excites both C and Aδ-fibers in the guinea pig (15), and we were able to demonstrate for the first time that it also stimulates the cough reflex in guinea pigs in vivo. Moreover, in keeping with our cough data, its excitatory effect on single fibers was unaffected by capsazepine. Although inhalation of chloride-deficient solutions induces cough in human subjects (20) we did not see an effect of such a stimulus in the present study. However, single-fiber studies in the guinea pig trachea have shown that low chloride is a relatively mild stimulus for both C and Aδ-fibers compared with capsaicin and hypertonic saline, producing low firing responses in only a small proportion of fibers (15). This may therefore account for the lack of cough response in the guinea pig.

In conclusion, we have shown that capsazepine inhibits cough evoked by capsaicin and citric acid. This does not reflect a nonspecific inhibitory action on airway sensory nerves, since hypertonic saline-induced cough was unaffected. These findings provide further evidence for the release by protons of an endogenous ligand for the capsaicin receptor in the guinea pig airway. They may also indicate that capsazepine may be useful as an antitussive agent.

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Exhibit AD

COUGH

Antitussive activity of iodo-resiniferatoxin in guinea pigs

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Background: Iodo-resiniferatoxin (I-RTX) has recently been described as an ultra potent antagonist of the transient receptor potential vanilloid-1 (TRPV1).
Methods: The ability of I-RTX to inhibit cough induced by inhalation of two putative TRPV1 stimulants (capsaicin and citric acid) was tested in non-anaesthetised guinea pigs.
Results: Pretreatment with I-RTX either intraperitoneally (0.03–0.3 μmol/kg) or by aerosol (0.1–3 μM) reduced the number of coughs produced by inhalation of citric acid (0.25 M) and capsaicin (30 μM) in a dose dependent manner. Capsazepine (CPZ) also reduced citric acid and capsaicin induced cough, but the activity of I-RTX was 10–100 times more potent than CPZ in all the experimental conditions tested.
Conclusions: I-RTX is a novel and potent antitussive drug which inhibits cough mediated by agents possibly acting via TRPV1 activation.

Cough is one of the most common reasons for medical consultation. In most cases, however, drugs currently available for antitussive treatment are only partially effective. In the search for safe and effective antitussive drugs it has been noted that, in some animal models and in humans, tussigenic agents stimulate a recently cloned ion channel belonging to the transient receptor potential family of channels.[1] Since this entity is activated by molecules with a vanilloid moiety it has been termed the vanilloid receptor-1 (VR1), and more recently reclassified as the transient receptor potential vanilloid-1 (TRPV1).[1 2]

TRPV1 is stimulated by several different agents including heat (43–52°C),[1] protons,[3 4] anandamide,[5] and other lipid derivatives.[6 7] Capsaicin, a well known stimulant of TRPV1, is frequently used in provocation tests to induce cough in experimental animal models and also in man.[8–10] Citric acid is also widely used for this purpose. The observation that capsazepine (CPZ), a relatively selective but low potency TRPV1 antagonist, reduced both capsaicin and citric acid induced cough suggested that TRPV1 is involved in the tussive response induced by these two agents.[11] A lowered threshold to capsaicin induced cough seems to be linked to the sex of the patient[12] and has been seen in patients with chronic inflammatory airway diseases including asthma[13] and chronic obstructive pulmonary disease (COPD).[14] TRPV1 therefore seems to have a role in the physiological activation of the cough reflex, as well as in the exaggerated cough response observed during certain pathological states.

On this basis it can be predicted that TRPV1 antagonists are of therapeutic value not only in the treatment of cough in patients with asthma and COPD, but also in patients with other inflammatory diseases including post viral cough and cough related to gastro-oesophageal reflux where upregulation of TRPV1 sensitivity has been implicated.[15] However, the TRPV1 antagonists currently available suffer from poor specificity or potency. For instance, ruthenium red selectively inhibits TRPV1 in a very narrow range of concentrations[16] whereas, because of its low potency, CPZ effectively blocks it only at high concentrations.[17] It has recently been reported that iodo-resiniferatoxin (I-RTX), the iodinated form of the ultra potent TRPV1 agonist resiniferatoxin,[18] behaves as a high affinity TRPV1 antagonist at the mouse and rat recombinant TRPV1.[19] We have confirmed this early observation by showing that, in a series of "typical" nociceptive or neurogenic inflammatory responses activated by capsaicin, I-RTX behaves as an ultra potent antagonist at the native rat and guinea pig TRPV1 and at the recombinant human TRPV1.[20]

The aim of the present study was to investigate whether I-RTX can reduce capsaicin and citric acid induced cough in guinea pigs and to compare its potency with that of CPZ.

METHODS

Animals

Male Dunkin-Hartley guinea pigs (250–350 g, Pampaloni, Italy) were acclimatised in cages at a mean (SD) temperature of 24 (0.5)°C) for 1 week after delivery and were allowed free access to water and standard rodent diet (Morini, Italy). All experiments complied with the national guidelines and were approved by the regional ethics committee.

Experimental set up

After acclimatisation to laboratory conditions, animals were individually placed in a transparent perspex box (20×10×10 cm, Vetrotecnica, Italy) ventilated with a constant airflow of 400 ml/min. The tussive agents (citric acid 0.25 M and capsaicin 30 μM) were nebulised via a mini-ultrasonic nebuliser (Ugo Basile, Italy). The particle size produced had an aerodynamic mass median diameter of 0.9 μm and the output of the nebuliser was 0.4 ml/min. The appearance of cough was detected by means of a tie clip microphone (Sony, Japan) and confirmed by the characteristic posture of the animal. The cough sounds were recorded and digitally stored. The number of elicited cough efforts was subsequently counted by a blinded observer.

Study protocols

All experiments were carried out at the same time of day starting at 09.00 hours. The guinea pigs were exposed to aerosols of either capsaicin or citric acid for 10 minutes to elicit cough. To evaluate the effects of aerosolised I-RTX on experimentally induced cough, guinea pigs inhaled various concentrations of I-RTX corresponding to 0.3, 1, and 3 μM in capsaicin trials and 0.1, 0.3, and 1 μM in citric acid trials. To evaluate the effects of aerosolised CPZ on capsaicin and citric acid induced cough, guinea pigs inhaled various

Abbreviations: ASIC, acid sensing ion channel; CPZ, capsazepine; I-RTX, iodo-resiniferatoxin; RAR, rapidly adapting receptor; TRPV1, transient receptor potential vanilloid-1

concentrations of CPZ (30, 100, and 300 μM) before exposure to each tussigenic agent. Both the I-RTX and CPZ inhalation times were set at 10 minutes. In experiments aimed at evaluating the effects of I-RTX injected intraperitoneally (ip) on induced cough, guinea pigs were administered 0.03, 0.1, and 0.3 μmol/kg I-RTX 15 minutes before both capsaicin and citric acid inhalation. To determine the effects of ip CPZ on induced cough the animals were given 0.3, 1, and 30 μmol/kg CPZ before capsaicin inhalation or 0.1, 0.3, and 1 μmol/kg CPZ before inhalation of citric acid. In all experiments the effects of ip or aerosol administration of the I-RTX and CPZ vehicles (control conditions) on induced cough were also determined. Each animal received only one dose of antagonist.

To detect any non-specific inhibitory properties of ip I-RTX on the cough reflex, the ability of hypertonic saline (7% sodium chloride, 1.2 M) to induce cough was also investigated. Hypertonic saline was administered for 10 minutes by aerosol 15 minutes after administration of the antagonist or its vehicle.

Drugs

Agents were obtained from the following companies: sodium chloride, citric acid, capsaicin, CPZ, (Sigma, Italy); I-RTX, (Tocris, UK). The stock concentrations of capsaicin (10 mM) and CPZ (10 mM) were prepared in 100% ethanol. The stock concentration of I-RTX (1 mM) was prepared in 100% DMSO.

Data analysis

Values are presented as mean (SE). Comparisons between groups were made by one way analysis of variance (ANOVA) and the Student's t test or the Bonferroni t test when appropriate. A p value of <0.05 was considered significant. A minimum of eight guinea pigs was used to test the effect of the vehicle or of each single dose of the drugs. The inhibitory potency of I-RTX and CPZ was compared using the dose of antagonist that produces 50% inhibition (ED_{50}).

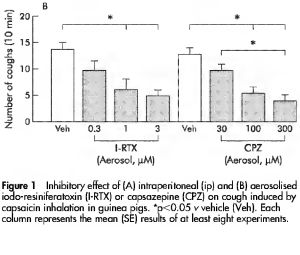

Figure 1 Inhibitory effect of (A) intraperitoneal (ip) and (B) aerosolised iodo-resiniferatoxin (I-RTX) or capsazepine (CPZ) on cough induced by capsaicin inhalation in guinea pigs. *$p<0.05$ v vehicle (Veh). Each column represents the mean (SE) results of at least eight experiments.

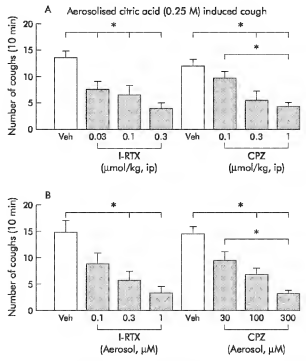

Figure 2 Inhibitory effect of (A) intraperitoneal (ip) and (B) aerosolised iodo-resiniferatoxin (I-RTX) or capsazepine (CPZ) on cough induced by citric acid inhalation in guinea pigs. *$p<0.05$ v vehicle (Veh). Each column represents the mean (SE) results of at least eight experiments.

RESULTS

Independent of the route of administration, pretreatment with I-RTX and CPZ caused no obvious cough response in awake, freely moving guinea pigs. In no instance were inhalations of aerosolised CPZ or I-RTX followed by the appearance of even a few cough efforts. In contrast, inhalation of both capsaicin and citric acid (alone or in the presence of the CPZ or I-RTX vehicles) consistently caused a brisk tussive response in all animals tested.

The effects of pretreatment with I-RTX and CPZ on experimentally induced cough are shown in figs 1 and 2. Compared with control conditions, ip and aerosol administration of I-RTX and CPZ consistently reduced the number of coughs provoked by inhalation of both capsaicin and citric acid ($p<0.05$ in both sets of experiments). Furthermore, in all the experiments the inhibitory effects of I-RTX and CPZ on induced cough were dose dependent. Table 1 shows the ED_{50} values calculated after completion of the experiments. I-RTX administered either by aerosol or ip was significantly more potent than CPZ. Inhalation of hypertonic saline (7% sodium chloride, 1.2 M) provoked a significant increase in the number of coughs (fig 3). Pretreatment with the highest dose of I-RTX (0.3 μmol/kg ip) used in this study did not affect the number of coughs induced by hypertonic saline inhalation.

DISCUSSION

The results show that intraperitoneal and aerosol administration of I-RTX is effective in reducing the number of coughs evoked by inhalation of both capsaicin and citric acid in guinea pigs. A previous investigation showed that I-RTX is a potent compound in antagonising capsaicin induced

Table 1 ED_{50} values of iodo-resiniferatoxin and capsazepine in inhibiting cough induced by capsaicin and citric acid in guinea pigs

	Iodo-resiniferatoxin		Capsazepine	
	Aerosol (nM)	Intraperitoneal (nmol/kg)	Aerosol (nM)	Intraperitoneal (nmol/kg)
Capsaicin	284 (92)*	29 (9)*	34280 (867)	860 (27)
Citric acid	84 (19)*	16 (4)*	12840 (1621)	150 (6)

Data are mean (SE) of at least eight experiments.
*p<0.01 v capsazepine.

contractions in isolated bronchial rings and strips of guinea pig urinary bladder.[19] The contractile response produced by capsaicin in these tissues results from smooth muscle contraction secondary to tachykinin release from terminals of primary sensory neurons following stimulation of TRPV1. We suggest that the inhibitory effect of I-RTX on capsaicin induced cough in guinea pigs is also caused by its ability to antagonise the activation of TRPV1. It should be emphasised, however, that the pharmacological characterisation of I-RTX as a TRPV1 antagonist is far from complete. Previous evidence indicated that I-RTX is a powerful antagonist at TRPV1 in vitro and in vivo.[14 20] However, later studies confirmed the high potency of I-RTX in vitro but not in vivo in capsaicin induced paw flinching in rats.[21] In this latter study I-RTX produced some excitatory effects at the highest doses. Likewise, minor excitation was produced by I-RTX when injected intraperitoneally to induce the writhing response.[20] It is possible that a minor agonistic component is retained by I-RTX and is unmasked when the drug is administered locally in a circumscribed environment (intraperitoneally, subcutaneously), but not following systemic administration.[19 20] Thus, the possibility that I-RTX retains some agonistic activity and that an agonist-dependent desensitising effect on sensory nerves contributes to its sensory neuron blocking activity cannot be completely discounted. In contrast, in none of our experiments did I-RTX cause a tussive response. One possible explanation for this is that inhalation of the drug causes widespread diffusion of the molecules into the airways so that its minor (if any) agonistic activity is minimised while its antagonist potential is maintained.

Citric acid is thought to induce cough by its ability to donate protons that are powerful stimulants of different subpopulations of primary sensory neurons including the rapidly adapting receptors (RAR)—for example, the subtype that plays a major role in the tussive response.[22] Protons may stimulate neurons with C and A-δ fibres by activating different channels, two of which have been well characterised: (1) the TRPV1[3 4] and (2) the acid sensing ion channels (ASICs).[23] It has been suggested that RARs do not express TRPV1,[22] which suggests that ASIC ought to be responsible for the cough induced by citric acid. However, a previous study showed that CPZ was able to inhibit citric acid induced cough.[11] Since CPZ is not a high affinity antagonist of TRPV1, this finding may raise doubts as to its selectivity in inhibiting the cough response. Nevertheless, the findings of Lalloo *et al*[11] suggested that TRPV1 rather than ASIC is responsible for citric acid induced cough. The present findings strongly support this view as I-RTX, a TRPV1 antagonist chemically unrelated to CPZ, is also able to inhibit citric acid induced cough effectively. However, a small component of the cough response to citric acid remained even at the highest doses of both I-RTX and CPZ. This residual response might be due to stimulation by citric acid of a non-TRPV1 channel such as the ASIC. Alternatively, it may still be caused by TRPV1 activation due to partial blockade of the channel by the two antagonists.

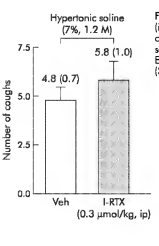

Figure 3 Effect of intraperitoneal (ip) iodo-resiniferatoxin (I-RTX) on cough induced by hypertonic saline inhalation in guinea pigs. Each column represents the mean (SE) of at least eight experiments.

Capsaicin induced cough was also not completely inhibited by I-RTX or CPZ. Since it is presumed that the cough response induced by capsaicin is entirely mediated by TRPV1, it is likely that the incomplete abolition of citric acid induced cough by CPZ and I-RTX is caused by the partial blockade of TRPV1 at the doses of the antagonists used in the present study. An alternative explanation is that splice variants such as those described in the rat[24 25] also occur in guinea pigs, and these variants could exhibit different affinities for different agonists. In addition, although the expression in recombinant systems suggests that the channel monomer is sufficient for the pore function of TRPV1, there is evidence that TRPV1 is capable of forming a specific ternary complex with phospholipase C and the neurotrophin trkA receptor.[26] These differences in assembling the channel may be tissue specific and may result in different affinities of antagonists.

Finally, recent electrophysiological observations[27] in single airway neurons of guinea pigs show that nerve activation following exposure to acid occurs in two different ways: a slowly inactivating mechanism present in C fibres which is TRPV1 dependent as it is blocked by CPZ and I-RTX, and a rapidly inactivating mechanism present in A-δ fibres which acts independently of TRPV1. The pharmacological observation that vanilloid receptor antagonism (present findings) inhibits most of the tussive response to citric acid[11] leads to the surprising conclusion that the TRPV1 resistant pathway has a minor role in acid induced cough in guinea pigs.

Another important observation of the present study is that I-RTX was several times more potent than CPZ in reducing the cough response to capsaicin and citric acid. In vitro assays in different mammalian species have shown that I-RTX has a very high potency towards TRPV1, being 100–1000 times more potent than CPZ.[19 20] This striking difference in vitro between I-RTX and CPZ has been confirmed in vivo in the mouse and rat where neurogenic plasma extravasation and nociceptive responses such as the writhing test[20] or the capsaicin pain test have been studied.[19] The finding that

I-RTX was 10–100 times more potent than CPZ in inhibiting experimentally induced cough is therefore in keeping with the results of previous in vivo investigations.[19 20] The observation that both CPZ and I-RTX were more effective (2–5-fold) in inhibiting cough induced by citric acid than cough induced by capsaicin further supports the view that citric acid cough is sensitive to TRPV1 antagonism.

The overall importance of TRPV1 in human cough is not fully understood. Available evidence suggests that TRPV1 is of considerable importance because stimuli that activate these channels are powerful tussive agents. In disease states the sensitivity of TRPV1 may be upregulated by proinflammatory mediators including bradykinin and nerve growth factor[26 29] which have an important role in cough and asthma.[30] The threshold dose of capsaicin to induce cough in patients with asthma and COPD[13 14] is lowered, and patients with gastro-oesophageal reflux often suffer from cough. A high affinity and selective TRPV1 antagonist is needed to show the pathophysiological role of TRPV1 in the cough experienced by these different groups of patients.

In conclusion, we have shown that I-RTX, a novel and ultra potent antagonist at the native rat and guinea pig TRPV1 and at the recombinant human TRPV1, inhibits cough mediated by agents possibly acting via TRPV1 stimulation. I-RTX can therefore be considered as an exemplar for the design of novel antitussive agents.

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